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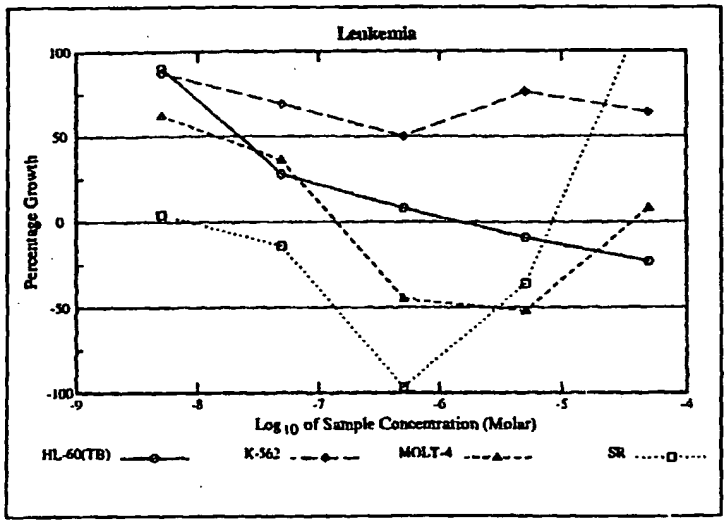
(54) Title: CYCLIC PEPTIDE ANTI-CANCER AGENTS AND METHODS



(57) Abstract: The present invention provides new types of anti-cancer agents. Methods of using the present anti-cancer agents are also provided.

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CYCLIC PEPTIDE ANTI-CANCER AGENTS AND METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority from U.S. Provisional Patent Application Serial No. 60/378,395, filed May 6, 2002, by Ghadiri, entitled "Cyclic Peptide Anti-Cancer Agents and Methods," the contents of which are hereby incorporated by reference in its entirety. This application is related to PCT Application No. PCT/US02/14329, filed May 6, 2002 and to U.S. Provisional Patent Application Serial No. 60/378,256, filed May 6, 2002, the contents of which are hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The field of the invention includes cancers, as well as the treatment and/or prophylaxis of cancer and/or tumors and compositions therefor.

BACKGROUND OF THE INVENTION

The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art, or relevant, to the presently described or claimed inventions, or that any publication or document that is specifically or implicitly referenced is prior art.

Cancer is a major disease that continues to be a leading cause of death at any age. In the United States alone, about half a million Americans die of cancer each year, totaling over 1500 people a day. One in four deaths in the U.S. are from cancer and it is the second leading cause of death in the U.S., behind heart disease. The exact causes of all cancers are not known, but links between certain activities such as smoking or exposure to carcinogens and the incidence of certain types of cancers and tumors have been shown by a number of researchers.

Various approaches to cancer include surgery, radiation, biological therapies (interferons, interleukins, tumor necrosis factor, colony-stimulating factors, monoclonal antibodies, and cancer vaccines), anticancer drugs (chemotherapy), or a combination of these treatments. Currently, chemotherapy and biological therapy are two important methods used in the treatment of cancer. Several types of chemotherapeutic and biologic agents have been shown to be effective against cancers and tumor cells, but not all types of cancers and tumors respond to these agents, and cancers can often reoccur. Unfortunately, many of these agents also destroy normal cells.

Despite advances in the field of cancer treatment the development of cytotoxic agents that have specificity for cancer and tumor cells while not affecting normal cells would be extremely desirable. Unfortunately, none have been found and instead agents that target especially rapidly dividing cells (both tumor and normal) have been used. Considerable efforts are underway to develop new agents for more potent and specific anti-cancer therapy, presenting effective and efficient cytotoxicity against tumor cells, with minimal interference with normal cell function, and the development of materials that would target tumor cells due to some specificity for them would be a breakthrough. Alternatively, materials that were cytotoxic to tumor cells while exerting mild effects on normal cells are also desirable. Accordingly, there is an urgent need for the development of novel, effective anti-cancer agents.

SUMMARY OF THE INVENTION

The present invention provides new, fast-acting cyclic peptide anti-cancer agents for treating and/or preventing cancer in a human or other animal. The present cyclic peptides are highly effective for many cancers. Cyclic peptides are fast acting, proteolytically stable and easy to synthesize. Other cyclic peptides of the invention do not have undesired toxicity against normal mammalian cells, for example, as measured by hemolysis of erythrocytes.

As used herein, the term "cancer" includes solid mammalian tumors as well as hematological malignancies. "Solid mammalian tumors" include cancers

of the head and neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin central nervous system; sarcomas of the soft tissue and bone; and melanoma of cutaneous and intraocular origin. The term "hematological malignancies" includes childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and chronic leukemia, plasma cell neoplasm and cancers associated with AIDS. In addition, a cancer at any stage of progression can be treated, such as primary, metastatic, and recurrent cancers. Information regarding numerous types of cancer can be found, *e.g.*, from the American Cancer Society (www.cancer.org), or from, *e.g.*, Wilson et al. (1991) Harrison's Principles of Internal Medicine, 12th Edition, McGraw-Hill, Inc. Both human and veterinary uses are contemplated.

As used herein the terms "normal mammalian cell" and "normal animal cell" are defined as a cell that is growing under normal growth control mechanisms (*e.g.*, genetic control) and displays normal cellular differentiation. Cancer cells differ from normal cells in their growth patterns and in the nature of their cell surfaces. For example cancer cells tend to grow continuously and chaotically, without regard for their neighbors, among other characteristics well known in the art.

The invention provides anti-cancer cyclic peptides and pharmaceutical compositions thereof wherein the cyclic peptides comprise a sequence of from four to about sixteen alternating D- and L-α-amino acids, or from three to ten β-amino acids, wherein the cyclic peptide does not have undesired activity against normal mammalian cells. Activity against cancer cells can be evaluated using assays and techniques known in the art. Activity against normal mammalian cells can also be measured using assays and techniques known in the art, for example, by the ability of peptides to cause hemolysis of mammalian red blood cells *in vitro*. Such cyclic peptides and pharmaceutical compositions can be used for treating or preventing cancer in a mammal.

Cyclic peptides may have a lethal dose (LD_{100}) or a 50% inhibitory dose (ED_{50}) at which substantially no cancer cells grow *in vitro* that is less than the peptide concentration needed to cause 50% hemolysis of normal mammalian red blood cells. For example, the LD_{100} or ED_{50} can be less than half the peptide concentration needed to cause 50% hemolysis of normal mammalian red blood cells. In other embodiments, the LD_{100} or ED_{50} can be less than one fifth to less than one quarter the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells. In other embodiments, the LD_{100} or ED_{50} is less than at least one twentieth to less than at least one tenth the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells.

While not intending to be bound by any particular theory or mechanism of action, the cyclic peptides of the invention are believed to self-assemble into supramolecular structures within or by association with cancer cell membranes. Such supramolecular structures can be nanotubes, barrels of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof. These types of supramolecular structures can selectively induce cancer cell membrane depolarization or disruption while not depolarizing or disrupting normal cell membranes to a substantial or undesired degree. Further, the supramolecular structures can selectively induce cancer cell membrane lysis while not lysing normal cell membranes to a substantial or undesired degree.

Cyclic peptides of the invention can have a plurality of amino acids having side chains with affinity for biomolecules integral to cancer cell membranes. Such biomolecules can facilitate selective assembly of the cyclic peptides into supramolecular structures within cancer cell membranes. Most normal mammalian or other animal cells do not have the same biomolecules within their cellular membranes. While not intending to be bound by any particular theory or mechanism of action, cyclic peptides of the invention are believed to selectively associate with cancer cell membranes over normal mammalian or other animal cell membranes.

The cyclic peptides can have a half-life in the bloodstream of the mammal of about six hours or less, at an amount that is effective against cancer.

Such an effective amount is an amount of the cyclic peptide that is sufficient to induce death or lysis of cancer cells without inducing an undesired amount of death or lysis of normal mammalian cells, for example. The cyclic peptides of the invention preferably induce substantially no hemolysis of normal red blood cells at anti-cancer effective doses.

In some embodiments, the cyclic peptides of the invention can be administered in multiple doses over a period of one to seven days. An effective amount of the present cyclic peptides is about 0.1 mg/kg to about 100 mg/kg of body weight, alternatively about 0.5 mg/kg to about 50 mg/kg of body weight, about 1.0 mg/kg to about 30 mg/kg of body weight, and other amounts set forth herein.

The cyclic peptides of the invention generally have about 25% to about 88% D- and/or L-polar amino acids. In some embodiments, the percentage of polar amino acids can be from about 33% or 50% to about 65% or 88% of the total number of D- and L-amino acids. Thus, for example, an eight residue cyclic peptide of the invention can have at least one, alternatively, two to seven polar D- and L-amino acids. Other eight residue cyclic peptides will have three to five polar D- and L-amino acids for example. In some embodiments, for example, six residue cyclic peptides of the invention can have two to five polar D- and L-amino acids. Other six residue cyclic peptides may have three to four polar D- and L-amino acids. At least one of these polar D- or L-amino acids may be adjacent to at least one other polar D- or L-amino acid. Alternatively, at least one polar D- or L-amino acid may be adjacent only to nonpolar D- or L-amino acids.

A variety of polar amino acids are available to one of skill in the art. Examples of polar D- and L-α-amino acids that can be utilized in the peptides of the invention include the D- or L-enantiomers of serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, histidine, arginine, lysine, hydroxylysine or ornithine.

The cyclic peptides of the invention generally have about 25% to about 88% D- and/or L-ionizable amino acids. In some embodiments, the percentage of ionizable amino acids can be from about 33% or 50% to about 65% or 88% of

the total number of D- and L-amino acids. Thus, for example, a six or eight residue cyclic peptide can have at least one, or alternatively two or three or more ionizable D- and/or L-amino acids. In other embodiments, the cyclic peptides of the invention can have four to six ionizable D- and/or L-amino acids. Such an ionizable D- or L-amino acid can be adjacent to at least one other polar or ionizable D- or L-amino acid. Alternatively, the cyclic peptides of the invention can have at least one ionizable D- or L-amino acid that is adjacent only to nonpolar D- or L-amino acids.

Many types of ionizable amino acids are available to one of skill in the art and the invention contemplates all such ionizable amino acids. Examples of ionizable D- and/or L-amino acids include the D- or L-enantiomers of arginine, aspartic acid, glutamic acid, histidine, lysine, hydroxylysine or ornithine.

The cyclic peptides of the invention can have nonpolar D- and/or L-amino acid residues. The cyclic peptides of the invention generally have about 12% to about 75% D- and/or L-nonpolar amino acids. In some embodiments, the percentage of nonpolar amino acids can be from about 50% to about 67% or 75% of the total number of D- and/or L-amino acids. Thus, for example, an eight residue cyclic peptide of the invention can have at least one, alternatively, two to seven nonpolar D- and/or L-amino acids. Other eight residue cyclic peptides may have three to five nonpolar D- and/or L-amino acids. In some embodiments, for example, six residue cyclic peptides of the invention have two to five nonpolar D- and/or L-amino acids. Other six residue cyclic peptides may have three to four nonpolar D- and/or L-amino acids. At least one of these nonpolar D- and/or L-amino acids may be adjacent to at least one other nonpolar D- and/or L-amino acid. Alternatively, at least one nonpolar D- or L-amino acid may be adjacent only to polar D- or L-amino acids.

Many types of nonpolar amino acids are available to one of skill in the art and the invention contemplates all such nonpolar amino acids. Examples of nonpolar amino acids include the D- and L-enantiomers of alanine, valine, isoleucine, leucine, methionine, norleucine, phenylalanine, tyrosine or tryptophan.

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In addition to the amino acids found naturally in proteins, other naturally occurring amino acids may be used, as well as non-naturally occurring and synthetic amino acids.

In one embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula I:

$$\begin{bmatrix} (Y_1)_p\text{-}(X_1)_m\text{-}(Y_2)_p\text{-}(X_2)_p\text{-}(Y_3)_p\text{-}(X_3)_p\text{-}(Y_4)_p\text{-}(X_4)_p\text{-}(Y_5)_p\text{-}(X_5)_p \\ (X_{10})_p\text{-}(Y_{10})_p\text{-}(X_9)_p\text{-}(Y_9)_p\text{-}(X_8)_p\text{-}(Y_8)_p\text{-}(X_7)_p\text{-}(Y_7)_p\text{-}(X_6)_p\text{-}(Y_6)_p \end{bmatrix}$$

I

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid; and

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In another embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula II:

$$\begin{bmatrix} (\text{D-}X_1\text{ -L-}X_2)_m - (\text{D-}Y_1 - \text{L-}Y_2)_p - (\text{L-}X_3 - \text{D-}X_4)_p - (\text{L-}Y_3 - \text{D-}Y_4)_p \\ (\text{L-}Y_8 - \text{D-}Y_7)_p - (\text{D-}X_8\text{ -L-}X_7)_p - (\text{D-}Y_6 - \text{L-}Y_5)_p - (\text{L-}X_6 - \text{D-}X_5)_p \end{bmatrix}$$

II

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, and Y_8 is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In yet another embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula III:

$$\begin{bmatrix} (X_1)_p\text{-}(X_2)_p\text{-}(X_3)_p\text{-}(X_4)_m\text{-}(X_5)_p\text{-}(X_6)_p\text{-}(X_7)_p\text{-}(X_8)_p\text{-}(X_9)_p\text{-}(X_{10})_p \\ (Y_{10})_p\text{-}(Y_9)_p\text{-}(Y_8)_p\text{-}(Y_7)_p\text{-}(Y_6)_p\text{-}(Y_5)_p\text{-}(Y_4)_p\text{-}(Y_3)_p\text{-}(Y_2)_p\text{-}(Y_1)_p \end{bmatrix} \quad \textbf{III}$$

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In yet another embodiment, the cyclic peptide has an amino acid sequence of formula IVa or IVb:

$$\text{D-}X_1 - (\text{L-}X_2 - \text{D-}X_3)_n - (\text{L-}Y_1 - \text{D-}Y_2)_m - \text{L-}Y_3 \qquad \text{IVa}$$

$$\text{L-}X_1 - (\text{D-}X_2 - \text{L-}X_3)_n - (\text{D-}Y_1 - \text{L-}Y_2)_m - \text{D-}Y_3 \qquad \text{IVb}$$

wherein:

n is an integer ranging from 0 to 4;

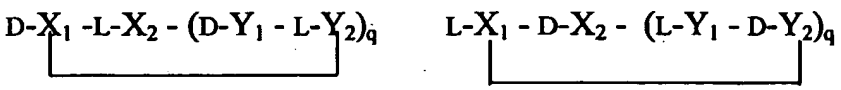
m is an integer ranging from 1 to 7;

X_1, X_2 and X_3 are each separately a polar amino acid;

Y_1, Y_2 and Y_3 are each separately nonpolar amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In another embodiment, the cyclic peptide has an amino acid sequence of formula Va or Vb:



Va Vb

wherein:

q is an integer ranging from 2 to 7;

X_1 and X_2 are separately polar amino acids;

Y_1 and Y_2 are separately nonpolar amino acids.

The claimed cyclic peptides of the above formulae, however, exclude those composed entirely of nonpolar amino acids.

Cyclic peptides often have, for example, from four to about sixteen D- and L-α-amino acids. In other embodiments, the cyclic peptides have about six to about ten or twelve D- and L-α-amino acids. In still other embodiments, cyclic peptides of about six or eight D- and L-α-amino acids are employed.

The pharmaceutical compositions of the invention can include an effective amount of at least one of the cyclic peptides of the invention, or two or more different cyclic peptides of the invention. These compositions also include a pharmaceutically effective carrier.

According to the invention, the cyclic peptides need not be made from D- or L-α-amino acids and can alternatively have a sequence of from three to about ten homochiral β-amino acids. Such β-amino acids are available to one of skill in the art. Beta amino acids can be substituted at the α- or β-carbons by one to two substituents. Mono-substituted beta amino acids of either S or R chirality can be employed for the construction of cyclic β-peptides, provided that the cyclic beta peptide is homochiral. Di-substituted β-amino acids employed in the

homochiral β-peptides of the present invention have the relative R,R or S,S diastereomeric configuration. Cyclic peptides having β-amino acids generally have at least one β-amino acid with at least one polar side chain. Preferred β-peptides cause substantially no undesirable lysis of mammalian cells.

In one embodiment, the cyclic β-peptides of the invention can have an amino acid sequence of formula VI:

$$\left[\begin{array}{l}(Z_1)_p\text{-}(Z_2)_p\text{-}(Z_3)_p\text{-}(Z_4)_p\text{-}(Z_5)_p\text{-}(Z_6)_p\text{-}(Z_7)_p\text{-}(Z_8)_p\text{-}(Z_9)_p\text{-}(Z_{10})_p \\ (Z_{20})_p\text{-}(Z_{19})_p\text{-}(Z_{18})_p\text{-}(Z_{17})_p\text{-}(Z_{16})_p\text{-}(Z_{15})_p\text{-}(Z_{14})_p\text{-}(Z_{13})_p\text{-}(Z_{12})_p\text{-}(Z_{11})_p\end{array}\right]$$

VI

wherein:

each p is separately an integer ranging from 0 to 7;

each Z_1, Z_3, Z_5, Z_7, Z_9, Z_{11}, Z_{13}, Z_{15}, Z_{17}, and Z_{19} is separately a monosubstituted β-amino acid;

each Z_2, Z_4, Z_6, Z_8, Z_{10}, Z_{12}, Z_{14}, Z_{16}, Z_{18}, and Z_{20} is separately a disubstituted β-amino acid; and

wherein the cyclic β-peptide has a sequence of from three to about ten homochiral β-amino acids. The invention therefore also contemplates pharmaceutical compositions that include an effective amount of at least one cyclic peptide, or two or more different cyclic peptides, wherein these cyclic peptide(s) have a sequence of from three to about ten homochiral β-amino acids, for example, as provided by formula VI.

The invention also provides a method of treating or preventing a cancer in a mammal and other animals, which comprises contacting a cancer cell with a cyclic peptide comprising a sequence of from four to about sixteen amino acids, wherein the sequence has alternating D- and L-α-amino acids, in an amount sufficient to induce cancer cell death without inducing an undesirable amount of normal cell death. Such cyclic peptides can alternatively have a sequence of from three to about ten β-amino acids.

The invention further provides a method of identifying cyclic peptides selectively cytotoxic to a target cell-type comprising screening one or more cyclic peptides for induction of cell death in target cells without induction of substantial or undesired cell death in a second cell type. Such cyclic peptides may comprise, for example, an alternating D- and L- α amino acid sequence of between four and about sixteen amino acids. Alternatively, such cyclic peptides may have a sequence of from three to about ten homochiral β-amino acids.

The invention also provides a method of identifying cyclic peptides selectively cytotoxic to a target cell-type comprising: (a) making a combinatorial library of cyclic peptides, wherein each cyclic peptide in the combinatorial library comprises an alternating D- and L- α amino acid sequence of between four and about sixteen amino acids; and (b) screening cyclic peptides from the combinatorial library for induction of cell death in target cells without induction of substantial or undesired cell death in a second cell type. Alternatively, such cyclic peptides may have a sequence of from three to about ten homochiral β-amino acids. The library can be used to generate single cyclic peptides or mixtures of cyclic peptides. Mixtures of cyclic peptides that show anti-cancer activity can then be further screened to identify one or more anti-cancer cyclic peptides in one or more of the mixtures, which can then be isolated or synthesized and re-tested for induction of cell death in target cancer cells without induction of substantial or undesired cell death in a second cell type.

The invention further provides a method of identifying cyclic peptides selectively cytotoxic to a target cell-type. This method involves the step of rationally designing at least one cyclic peptide comprising an alternating D- and L- α amino acid sequence of between four and sixteen amino acids. Alternatively, such cyclic peptides may have a sequence of from three to about ten homochiral β-amino acids. The method further involves screening such rationally designed cyclic peptides for induction of cell death in target cells without induction of substantial or undesired cell death in a second cell type.

Rationally designing a cyclic peptide can involve identifying at least one effective cyclic peptide from a combinatorial library that can induce cell death in target cells without inducing substantial or undesired cell death in a second cell type and exchanging at least one amino acid for a different amino acid in the alternating D- and L- α amino acid sequence of the effective cyclic peptide to generate a rationally designed cyclic peptide. The rationally designed cyclic peptide can then be screened for induction of cell death in target cells without inducing substantial or undesired cell death in a second cell type. Alternatively, such cyclic peptides may have a sequence of from three to about ten homochiral β-amino acids.

The target cell-type can be a cancer cell and the second cell type can be a normal mammalian cell, for example, a mammalian red blood cell. Cell death of the second cell type can be detected by detecting hemolysis, for example.

The method can further include screening a third cell type, for example, by determining whether a peptide induces substantial cell death in the third cell type. The method can also include determining the minimum inhibitory dose at which a peptide can kill substantially all, or inhibit the growth, of the target cells.

Assays can be performed *in vitro* by separately contacting a peptide with the target cell type and with other cell types (e.g., a second or third or other cell type). Alternatively, assays can be performed *in vivo* by administering at least one peptide to a test animal comprising the target cell type and another cell type or types and determining whether the peptide is toxic to the target cell type but does not have substantial or undesired toxicity to another cell type or types. Such a method can also include determining whether the peptide adversely affects the health of the animal. Determining whether the peptide adversely affects the health of the animal can include examining the bodily fluids or the anatomy of the animal by pathological or histological methods.

The invention further provides a method of identifying cyclic peptides capable of selective association with one or more target biomolecules on a selected cell surface, comprising contacting a solution of cyclic peptides, each peptide comprising between four to about sixteen amino acids in an alternating

D- and L-α amino acid sequence, or a peptide comprising from three to about ten β-amino acids, with the target biomolecule(s) and determining, for example, whether the peptides spontaneously assemble into a supramolecular structure that selectively associates with the biomolecule(s). The target biomolecule can be displayed, for example, on the surface of a living cell or on the surface of a liposome. Alternatively, the peptide can be contacted with the target biomolecule(s). The method can further include determining the structure of the peptides that spontaneously assemble into the supramolecular structure that selectively associates with the biomolecule(s).

The present invention also provides methods of evaluating or confirming therapeutically effective dosages for treating a cancer with a cyclic peptide having an amino acid sequence of alternating D- and L-α-amino acids, or a cyclic peptide comprising from three to about ten β-amino acids, that includes determining the LD_{100} or ED_{50} of the cyclic peptide at which substantially no cancer cells grow or survive *in vitro*.

The present invention also provides a composition comprising one or more of the present cyclic peptides with one or more other anti-cancer agents.

Mammals and other animals including birds may be treated by the methods and compositions described and claimed herein. Such mammals and birds include humans, dogs, cats, and livestock, for example, horses, cattle, sheep, goats, chickens, turkeys and the like.

The invention therefore provides a pharmaceutical composition for treating, inhibiting or preventing growth of a cancer cell in an animal comprising a cyclic peptide in an amount effective to treat or prevent a target cancer in the animal, and a pharmaceutically acceptable carrier, wherein the cyclic peptide comprises a sequence of from 4 to about 16 alternating D- and L-α-amino acids or a sequence of three to about ten β-amino acids. Many of the cyclic peptides provided herein, for example, cause substantially no hemolysis of non-cancerous mammalian red blood cells.

The invention also provides a method for treating, inhibiting or preventing growth of a cancer cell in an animal comprising contacting a target

cancer cell with a cyclic peptide comprising a sequence of from four to about sixteen amino acids, wherein the sequence has alternating D- and L-α-amino acids, in an amount sufficient to induce target cancer cell death without inducing an undesirable amount of non-cancerous mammalian cell death.

The invention further provides a method for treating, inhibiting or preventing growth of a cancer cell in an animal comprising contacting target cancer cell with a cyclic peptide comprising a sequence of from three to about ten β-amino acids, in an amount sufficient to induce target cancer cell death without inducing an undesirable amount of non-cancerous mammalian cell death.

The invention further provides a method of identifying a cyclic peptide selectively cytotoxic to a target cancer cell-type comprising: (a) contacting said target cancer cell-type with a test cyclic peptide comprising a sequence of from four to about sixteen alternating D- and L- α amino acids, or a sequence of three to about ten β-amino acids; and (b) determining whether said test cyclic peptide induces cell death of said target cancer cell-type without inducing substantial or undesired cell death in a second cell type.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides a schematic drawing of the self-assembly of the present cyclic peptides into nanotubules. An eight-residue cyclic D, L-α-peptide with alternating D- and L-amino acids is depicted to the left, with emphasis on its flat ring-shaped conformation. Side chains (R) decorate the outside surface of the cyclic peptide. Upon self-assembly into a tube, a series of cyclic peptides align and undergo inter-peptide hydrogen bonding to form a tubular structure referred to herein as a nanotube (center). Self-assembly is directed by inter-subunit backbone-backbone hydrogen bonding resulting in a β-sheet-like open-ended hollow tubular supramolecular structure. This β-sheet like hydrogen bonding pattern is shown to the right. For clarity most side chains are omitted.

Figure 2 illustrates the modes of permeation that are accessible to peptide supramolecular structures. Depending on the composition and sequence

of amino acids employed in the cyclic peptides, supramolecular structures can interact with the membranes of cells through (a) pores, (b) barrel stave strictures, (c) carpet-like structures, or (d) alternate modes of action. Cyclic peptides are depicted as ring structures. Note that in Figure 2b, the hydrophobic tails of the membrane lipids interact with hydrophobic portions of the nanotubes, whereas in Figure 2d the hydrophilic heads of the membrane lipids interact with hydrophilic portions of the nanotubes.

Figure 3 provides a plot of the apparent proton transport (Figure 3a) and the carboxyfluoroscein release (Figure 3b) mediated by peptide SEQ ID NO:11 (cyclo-[Gln-D-Lys-(Trp-D-Leu)$_2$-Trp-D-Lys-]), as expressed in fractional fluorescence changes as a function time. For Figure 3b, the peptide was added at about 100 seconds, and the detergent triton X-100 was added at about 200 seconds.

Figure 4 provides an attenuated total reflectance (ATR) infrared (IR) spectra for peptide SEQ ID NO:11 (cyclo-[-Gln-D-Lys-(Trp-D-Leu)$_2$-Trp-D-Lys-] in DMPC multibilayers. The solid trace indicates absorbance of parallel-polarized light; the dashed trace provides absorbance of perpendicular-polarized light.

Figure 5 provides a structural comparison of supramolecular structures composed of: (a) cyclic β-tetrapeptides, and (b) cyclic D, L-α-octapeptides. This figure illustrates that, due to the unidirectional arrangement of the polar backbone amide groups, cyclic β-tetrapeptide supramolecular structures may possess a macrodipole moment reminiscent of an α-helix, while cyclic D, L-α-octapeptide supramolecular structures will, under most circumstances, not have such a net dipole moment. For clarity most side chains are omitted from the nanotube structures depicted in Figure 5a and 5b.

Figure 6 provides a thin section electron microscopy image of untreated *S. aureus* (ATCC 25923) displaying a normal intact membrane.

Figure 7 provides a thin section electron microscopy image of *S. aureus* (ATCC 25923) after exposure to 2XMIC concentration of cyclo[KSKWLWLW] for 2 hours. The image provides direct visualization of the membrane mode of

action. Arrows denote abnormal membrane structures caused by the peptide action.

Figure 8 provides a thin section electron microscopy image of *S. aureus* (ATCC 25923) after exposure to 2xMIC concentration of cyclo[KSKWLWLW] for 2 hours. The image provides direct visualization of the membrane mode of action. Arrows denote abnormal membrane structures caused by the peptide action.

Figure 9 illustrates the toxicity of peptides administered via intraperitoneal route. In Figure 9A, the mice were injected with varying concentrations of cyclo[RRKWLWLW]HCl. In Figure 9B, the mice were injected with varying concentrations of cyclo[KQRWLWLW]HCl. Mice were monitored over a period of 14 days for activity and mortality. Four mice per dose were used in each experiment.

Figure 10 illustrates the toxicity of peptides administered via intraperitoneal route. In Figure 10A, the mice were injected with varying concentrations of cyclo[KSKWLWLW]HCl. In Figure 10B, the mice were injected with varying concentrations of cyclo[KKLWLW]HCl. Mice were monitored over a period of 14 days for activity and mortality. Four mice per dose were used in each experiment.

Figure 11 provides a graph indicating the toxicity of peptide SEQ ID NO:12 in MOLT-4 (■) and HL-60 (TB) (♦) cancer cells. The graph shows the percent growth after 48 hours as compared to peptide SEQ ID NO:12 concentration.

Figure 12 provides the *in vitro* testing results for the SEQ ID NO:12 peptide in a variety of cancel lines.

Figures 13a - i provide dose response curves for the SEQ ID NO:12 peptide in a variety of cancel lines.

Figure 14 provides graphs of mean Log_{10} GI30, TGI and LC50 concentrations for the SEQ ID NO:12 peptide in a variety of cancel lines.

Figure 15 provides additional dose response curves for the SEQ ID NO:12 peptide in a variety of cancel lines..

Figure 16A and B provides a chart illustrating the concentration (moles/liter) of the indicated cyclic peptide that inhibits 50% (GI50) of cancer cell growth. As illustrated, a large variety of cancer cell lines were tested. Cyclic peptides [KSKKLWLW] (SEQ ID NO:149), [RHKHRWLW] (SEQ ID NO:151), [KRKWLW] (SEQ ID NO:125), and [KSKWLW] (SEQ ID NO:126) were effective against many cancer cell lines when used at micromolar concentrations. Cyclic peptides [KHKHFLWL] (SEQ ID NO:72) and [SEKHKLWW] (SEQ ID NO:157) were highly effective against selected cancer cell types when used at micromolar or 0.1 micromolar concentrations. Figure 16A provides the beginning of the chart while Figure 16B provides a continuation of the chart.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides small cyclic peptides and compositions that can quickly and selectively kill or inhibit growth of cancer cells without substantial toxicity toward normal cells. The present invention includes cyclic peptides, and pharmaceutical compositions comprising cyclic peptides, with either a sequence of alternating D-, and L-α-amino acids, or a sequence of β-amino acids, that can sample flat, ring-shaped conformations. Such ring-shaped conformations project the amino acid side chains of the cyclic peptides away from the center of the ring and orient the amide backbone approximately perpendicular to the plane of the ring structure. While not intending to be bound by any particular theory or mechanism of action, it is believed that under conditions that favor hydrogen bonding, for example, such as side chain charge neutralization through interactions with cell membrane constituents and/or contact with low dielectric constant environments of cell membranes, the cyclic peptides can self-assemble via intermolecular hydrogen bonding to form supramolecular structures. Cyclic peptides that simply contain one or more D-amino acids do not adopt a flat ring-shaped conformation and do not have the backbone conformation needed for self-assembly of the cyclic peptide into supramolecular structures.

Small changes in amino acid sequence of a cyclic peptide can be amplified into large differences at the supramolecular level. Thus, changes in the structure of a cyclic peptide may constrain peptide interaction and limit formation of supramolecular structures to particular cellular membranes that have particular membrane constituents, membrane partitioning properties, uptake properties, and the like.

While not intending to be bound by any particular theory or mechanism of action, another feature of the present self-assembling peptide supramolecular structures is believed to be the potential for a given cyclic peptide to form a number of diastereomeric nanotube assemblies. This property stems from the fact that backbone-backbone hydrogen bonding are believed primarily to direct the self-assembly of the nanotube structure. Differently stacked subunits can give rise to topoisomeric supramolecular structures that share the same or nearly the same tubular β-sheet-like hydrogen bonded backbone structure.

By varying the peptide sequence while retaining the cyclic D- and L-α-peptide backbone, or the cyclic β-peptide backbone, a multitude of cyclic peptides can quickly be screened or evaluated for the ability to selectively target and assemble in cancer cell membranes and exert anti-cancer activity by increasing cancer cell membrane permeability.

The term "amino acid," includes the residues of the natural α-amino acids (e.g. Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Hyl, Hyp, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val) in D or L form, as well as β-amino acids, synthetic and unnatural amino acids. Many types of amino acid residues are useful in the cyclic peptides and the invention is not limited to natural, genetically-encoded amino acids. Examples of amino acids that can be utilized in the cyclic peptide described herein can be found, for example, in Fasman, 1989, CRC Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Inc., and the references cited therein. Another source of a wide variety of amino acid residues is provided by the website of RSP Amino Acids Analogues, Inc. (www.amino-acids.com).

The term "mammal," as used herein, refers to an animal, such as a warm-blooded animal, which is susceptible to or has a cancer. Mammals include cattle, buffalo, sheep, goats, pigs, horses, dogs, cats, rats, rabbits, mice, and humans. Also included are other livestock, domesticated animals and captive animals. The term "farm animals" includes chickens, turkeys, fish, and other farmed animals.

While not intending to be bound by any particular theory or mechanism of action, as used herein, a "nanotube" or "nanotubule" is a small tubule that may spontaneously form from the cyclic peptides of the present invention. The present cyclic peptides are believed to stack to form supramolecular structures composed of nanotubes. Hydrogen bonding between cyclic peptides helps to drive the self-assembly of the supramolecular structures from the cyclic peptide and, after formation, acts to stabilize the structure. Each nanotube has a pore in the center of the tube that is surrounded by the series of peptide backbones of the stacked cyclic peptides that form the nanotubes. The size of the pore depends upon the number of amino acids in the cyclic peptides that form the nanotube. In general, ions, sugars, and other small molecules can travel through the pores of the present nanotubes. Larger molecules can also flow the pores of nanotubes formed from larger cyclic peptides and supramolecular structures formed of aggregates of nanotubes. For example, although not intending to be bound by any particular theory or mechanism of action, in some embodiments the supramolecular structure is thought to be a barrel-like structure composed of clusters of nanotubes. In other embodiments, the supramolecular structure is thought to be a "carpet" or "carpet-like" arrangement of nanotubes. A "carpet" or "carpet-like" arrangement of one or more nanotubes is where the nanotube(s) adopt orientations that can be approximately or somewhat parallel to the plane of the membrane structure. However, when in such a carpet-like arrangement, the nanotubes can assume other orientations relative to the plane of the membrane. For example, the nanotubes may be situated on the surface of the membrane, or may be partially or fully contained within the interior of the membrane. In one example, the carpet-like nanotubes can be oriented with a tilt angle of up to

approximately 70±5° from the membrane normal. These carpet or carpet-like arrangements may be formed because the contemplated cyclic peptides can possess clusters of hydrophilic and hydrophobic residues, that upon aggregation can form supramolecular structures with, for example, a hydrophilic side or face and a hydrophobic side or face. A hydrophilic face of a nanotube can, for example, be positioned so that it is in contact with the hydrophilic portions of the membrane or with the aqueous environment. A hydrophobic face of a nanotube can, for example, be in contact with the hydrophobic portions of the membrane. See Figure 2A-D.

The term "peptide" as used herein includes a sequence of from four to sixteen amino acids residues in which the α-carboxyl group of one amino acid is joined by an amide bond to the main chain (α- or β-) amino group of the adjacent amino acid. The peptides provided herein for use in the described and claimed methods and compositions are cyclic. Peptide sequences specifically recited herein are written with the amino terminus on the left and the carboxy terminus on the right. However, where the peptides are shown in cyclic form, the first amino acid in the sequence is arbitrarily chosen. Moreover, for formulae of cyclic peptides where the sequence extends onto two lines, the sequence on the second line extends from the N-terminal side on the right to the C-terminal side on the left.

While not intending to be bound by any particular theory or mechanism of action, according to the present invention, "supramolecular structures" are multi-subunit structures, e.g. nanotubes, barrels and carpets of nanotubules, which are believed to be formed through "noncovalent" assembly of cyclic peptides. Supramolecular structures may be contrasted with molecular or polymeric systems that are the product of covalent bond formation between reactants or monomers. The proposed peptide supramolecular structures are thermodynamically controlled assemblies that can undergo reversible structural assembly and disassembly. Such assembly-disassembly will depend, for example, on the environment, subunit structure, side group selection, side group interaction, and the nature and combination of noncovalent forces operating on

the system. In contrast, covalent polymeric structures have been used to design kinetically stable structures rather than structures that assemble and dissemble in response to the environment. Hence, one attractive feature of the present compositions containing peptides that can form supramolecular structures is their ability to select amongst various cell membrane types. Such selection is driven by favorable thermodynamic forces determined by the composition of the cyclic peptide relative to the cell membrane environment and the molecular and/or supramolecular constituents of the cell membrane.

The term "substantially no" with reference to self-assembly, hemolysis, toxicity or cellular lysis, or the like, means that little or no self-assembly, hemolysis, toxicity, cellular lysis or the like is present at the tested or desired peptide dosage or concentration. By way of example, "substantially no" hemolysis can mean that less than about 20%, alternatively less than 15% or less than 10%, or no detectable, hemolysis at the tested or desired peptide dosage or concentration has occurred. Similarly, "substantially no" toxicity or lysis can mean that less than about 20%, alternatively less than 15% or less than 10%, or no detectable, toxicity or lysis at the tested or desired peptide dosage or concentration has occurred. In other embodiments, "substantially no" hemolysis, toxicity or lysis means that less than about 5%, or no detectable, hemolysis, toxicity or lysis, etc., at the tested or desired peptide dosage or concentration has occurred.

The term "therapeutically effective amount" is that amount sufficient to control cancer growth. A therapeutically effective amount reduces the size of the cancer in the affected mammal by at least about 20%, by at least about 40%, by at least about 60%, or by at least about 80% relative to untreated subjects. In one example, a therapeutically effective amount reduces the size of the cancer in the affected mammal by at least about 90% or more. These percentages refer to a decrease in the size of the cancer found in the mammal relative to untreated subjects. While not intending to be bound by any particular theory or mechanism of action, a "therapeutically effective amount" may also be that amount of cyclic peptide needed to permeabilize or depolarize the cellular

membrane of the cancer. Alternatively, the term "therapeutically effective amount" is that amount needed to control the growth of, or kill, a cancer cell. An effective amount of the therapeutic agent used to control the cancer cell can vary according to factors such as the type of cancer, the amount of cancer already present in the animal, the age, sex, and weight of the mammal, and the ability of the cyclic peptides of the present invention to control cancer growth in the mammal.

Therapeutically effective amounts of the peptide and peptide compositions can also be used to prevent cancer, including preventing a recurrence of cancer.

Peptides, Peptide Variants, and Derivatives Thereof

The present invention provides cyclic peptides and compositions including cyclic peptides that have an amino acid sequence of alternating D- and L-amino acids that is between four to about sixteen, alternatively about six to about sixteen amino acids in length. Alternatively, the cyclic peptides of the present invention can have between three to about ten β-amino acids. In general, the cyclic D, L-α-peptides do not include the amino acids proline and glycine. According to the invention, β-amino acids can be substituted at the α- or β-carbons, or both. Mono-substituted β-amino acids of either S or R chirality can be employed for the construction of cyclic β-peptides, provided that the cyclic beta peptide is homochiral. Disubstituted β-amino acids employed in the present invention must have the relative R,R or S,S diastereomeric configuration, provided that the β-amino acid residues in a cyclic peptide structure are homochiral. Cyclic peptides having β-amino acids generally have at least one β-amino acid with at least one polar side chain.

While not intending to be bound by any particular theory or mechanism of action, the cyclic peptides of the present invention are believed to undergo self-assembly to form supramolecular structures that, upon assembly in or on a cancer cell membrane, can cause depolarization and/or permeablization and/or destabilization of the cancer cell membrane. In some cases, the cyclic peptides

cause lysis of the cancer cell. While not intending to be bound by any particular theory or mechanism of action, self-assembly into supramolecular structures is thought to occur by stacking of the cyclic peptides in an anti-parallel fashion or a parallel fashion with formation of β-sheet hydrogen bonds between adjacent cyclic peptides. However, it is believed that the preferred cyclic peptides do not readily or undesirably self-assemble into supramolecular structures in undesired mammalian cellular membranes as measured, for example, in an assay for toxicity in mammalian cells or hemolysis of mammalian red blood cells at tested or therapeutically effective doses.

Cyclic peptides of the present invention can be made from α-amino acids or β-amino acids. The amino acid sequence of the present cyclic peptides includes at least one polar amino acid in the case of D,L α-amino acid cyclic peptides, or at least one polar side chain in the case of cyclic β-peptides. The percentage of polar amino acids can range, for example, from about 25% or 33% to about 65% or 88%. However, in some embodiments a majority of the amino acids are polar. For example, the percentage of polar amino acids can be from about 50% to about 88% of the total number of amino acids. The exact number of polar and nonpolar amino acids depends on the size and the properties sought for a given cyclic peptide. In some embodiments, the sizes for the present cyclic peptides are about six to about ten D,L α-amino acids or about three to about ten β-amino acids. In other embodiments, the size for the present cyclic peptides is about six to about eight D,L α-amino acids or four to about six β-amino acids. Thus, for example, an eight residue cyclic peptide of the invention can have at least one, alternatively, two to seven polar D- and/or L-α-amino acids. Other eight residue cyclic peptides will have three to five polar D- and/or L-α-amino acids for example. Preferred eight residue cyclic peptides have three, four or five polar amino acids. In some embodiments, for example, six residue cyclic peptides of the invention can have two to five polar D- and/or L-α-amino acids. Other six residue cyclic peptides may have three to four polar D- and/or L-α-amino acids. At least one of these polar D- or L-α-amino acids may be adjacent

to at least one other polar D- or L-α-amino acid. Alternatively, at least one polar D- or L-α-amino acid may be adjacent only to nonpolar D- or L-α-amino acids. Beta peptides having about four to about eight β-amino acids may have, for example, about two to twelve polar side chains, depending on the level of α and β backbone substitution.

The cyclic D-L-α-peptides of the invention generally have about 25% to about 88% ionizable amino acid residues. In some embodiments, the percentage of ionizable amino acids can be from about 33% or 50% to about 65% or 88% of the total number of D- and/or L-amino acids. Thus, for example, a six or eight residue cyclic peptide can have at least one, or alternatively two or three or more ionizable D- and/or L-amino acids. In other embodiments, the cyclic peptides of the invention can have four to six ionizable D- and/or L-amino acids. Such an ionizable D- or L-amino acid can be adjacent to at least one other polar or ionizable D- or L-amino acid. Alternatively, the cyclic peptides of the invention can have at least one ionizable D- or L-amino acid that is adjacent only to nonpolar D- or L-amino acids. The cyclic β-peptides of the invention generally have about 25% to about 88% ionizable amino acid side chains. In some embodiments, the percentage of ionizable amino acid side chains can be from about 33% or 50% to about 65% or 88% of the total number of amino acid side chains. Thus, for example, a four to six residue cyclic β-peptide can have at least one, or alternatively two or three or more ionizable amino acid side chains. In other embodiments, the cyclic β-peptides of the invention can have four to six ionizable amino acid side chains.

The cyclic peptides of the invention can have nonpolar D- and/or L-amino acid residues. The number of non-polar amino acids chosen can vary as the size of the peptide varies and as the selected cancer cell membrane environment varies. The cyclic peptides of the invention generally have about 12% to about 75% D- and L-nonpolar amino acids. In some embodiments, the percentage of nonpolar amino acids can be from about 50% to about 67% or 75% of the total number of D- and L-amino acids. Thus, for example, an eight residue cyclic

peptide of the invention can have at least one, alternatively, two to seven nonpolar D- and/or L-amino acids. Other eight residue cyclic peptides may have three to five nonpolar D- and/or L-amino acids. In some embodiments, for example, six residue cyclic peptides of the invention have two to five nonpolar D- and/or L-amino acids. Other six residue cyclic peptides may have three to four nonpolar D- and/or L-amino acids. At least one of these nonpolar D- or L-amino acids may be adjacent to at least one other nonpolar D- or L-amino acid. Alternatively, at least one nonpolar D- or L-amino acid may be adjacent only to polar D- or L-amino acids. In general, the cyclic peptides do not include the amino acid proline or glycine, but certain cyclic peptides may have good activity even though proline or glycine is included.

According to the invention, β-amino acids can have non-polar side chains at the α- or β-carbons, or both. The number of non-polar amino acid side chains chosen can vary as the size of the peptide varies and as the selected cancer cell membrane environment varies. The cyclic β-peptides of the invention generally have about 12% to about 75% nonpolar amino acid side chains. In some embodiments, the percentage of nonpolar amino acid side chains can be from about 50% to about 67% or 75% of the total number of amino acid side chains. Thus, for example, an eight residue cyclic β-peptide of the invention can have at least one, alternatively, two to seven nonpolar amino acid side chains. Other eight residue cyclic β-peptides may have three to five nonpolar amino acid side chains. In some embodiments, for example, six residue cyclic β-peptides of the invention have two to five nonpolar amino acid side chains. Other six residue cyclic β-peptides may have three to four nonpolar amino acid side chains.

Amino acids used in the cyclic peptides can be genetically encoded amino acids, naturally occurring non-genetically encoded amino acids, or synthetic amino acids. Both L- and D-enantiomers of any of the above are utilized in the cyclic peptides. The amino acid notations used herein for the twenty genetically encoded L-amino acids and some examples of non-encoded amino acids are provided in Table 1.

Table 1

Amino Acid	One-Letter Symbol	Common Abbreviation
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamine	Q	Gln
Glutamic acid	E	Glu
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val
B-Alanine		BAla
2,3-Diaminopropionic acid		Dpr
A-Aminoisobutyric acid		Aib
N-Methylglycine (sarcosine)		MeGly
Ornithine		Orn
Citrulline		Cit
t-Butylalanine		t-BuA

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Amino Acid	One-Letter Symbol	Common Abbreviation
t-Butylglycine		t-BuG
N-methylisoleucine		MeIle
Phenylglycine		Phg
Cyclohexylalanine		Cha
Norleucine		Nle
Naphthylalanine		Nal
Pyridylalanine		
3-Benzothienyl alanine		
4-Chlorophenylalanine		Phe(4-Cl)
2-Fluorophenylalanine		Phe(2-F)
3-Fluorophenylalanine		Phe(3-F)
4-Fluorophenylalanine		Phe(4-F)
Penicillamine		Pen
1,2,3,4-Tetrahydro-isoquinoline-3-carboxylic acid		Tic
B-2-thienylalanine		Thi
Methionine sulfoxide		MSO
Homoarginine		Harg
N-acetyl lysine		AcLys
2,4-Diamino butyric acid		Dbu
P-Aminophenylalanine		Phe(pNH_2)
N-methylvaline		MeVal
Homocysteine		Hcys
Homoserine		Hser
E-Amino hexanoic acid		Aha
Δ-Amino valeric acid		Ava
2,3-Diaminobutyric acid		Dab

Certain commonly encountered amino acids that are not genetically encoded and that can be present in the cyclic peptides of the invention include,

but are not limited to, β-alanine (b-Ala) and other omega-amino acids such as 3-aminopropionic acid (Dap), 2,3-diaminopropionic acid (Dpr), 4-aminobutyric acid and so forth; α-aminoisobutyric acid (Aib); ε-aminohexanoic acid (Aha); δ-aminovaleric acid (Ava); methylglycine (MeGly); ornithine (Orn); citrulline (Cit); t-butylalanine (t-BuA); t-butylglycine (t-BuG); N-methylisoleucine (MeIle); phenylglycine (Phg); cyclohexylalanine (Cha); norleucine (Nle); 2-naphthylalanine (2-Nal); 4-chlorophenylalanine (Phe(4-Cl)); 2-fluorophenylalanine (Phe(2-F)); 3-fluorophenylalanine (Phe(3-F)); 4-fluorophenylalanine (Phe(4-F)); penicillamine (Pen); 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic); .beta.-2-thienylalanine (Thi); methionine sulfoxide (MSO); homoarginine (hArg); N-acetyl lysine (AcLys); 2,3-diaminobutyric acid (Dab); 2,3-diaminobutyric acid (Dbu); p-aminophenylalanine (Phe(pNH_2)); N-methyl valine (MeVal); homocysteine (hCys) and homoserine (hSer). Additional amino acid analogs contemplated include phosphoserine, phosphothreonine, phosphotyrosine, hydroxyproline, gamma-carboxyglutamate, hippuric acid, octahydroindole-2-carboxylic acid, statine, α-methyl-alanine, para-benzoyl-phenylalanine, propargylglycine, and sarcosine. Peptides that are encompassed within the scope of the invention can have any of foregoing amino acids in the L- or D- configuration, or any other amino acid known to one of skill in the art.

Amino acids that are substitutable for each other generally reside within similar classes or subclasses. As known to one of skill in the art, amino acids can be placed into different classes depending primarily upon the chemical and physical properties of the amino acid side chain. For example, some amino acids are generally considered to be hydrophilic or polar amino acids and others are considered to be hydrophobic or nonpolar amino acids. Polar amino acids include amino acids having acidic, basic or hydrophilic side chains and nonpolar amino acids include amino acids having aromatic or hydrophobic side chains. Nonpolar amino acids may be further subdivided to include, among others, aliphatic amino acids. The definitions of the classes of amino acids as used herein are as follows:

"Nonpolar Amino Acid" refers to an amino acid having a side chain that is uncharged at physiological pH, that is not polar and that is generally repelled by aqueous solution. Examples of genetically encoded hydrophobic amino acids include Ala, Ile, Leu, Met, Trp, Tyr and Val. Examples of non-genetically encoded nonpolar amino acids include t-BuA, Cha and Nle.

"Aromatic Amino Acid" refers to a nonpolar amino acid having a side chain containing at least one ring having a conjugated π-electron system (aromatic group). The aromatic group may be further substituted with substituent groups such as alkyl, alkenyl, alkynyl, hydroxyl, sulfonyl, nitro and amino groups, as well as others. Examples of genetically encoded aromatic amino acids include phenylalanine, tyrosine and tryptophan. Commonly encountered non-genetically encoded aromatic amino acids include phenylglycine, 2-naphthylalanine, β-2-thienylalanine, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, 4-chlorophenylalanine, 2-fluorophenylalanine, 3-fluorophenylalanine and 4-fluorophenylalanine.

"Aliphatic Amino Acid" refers to a nonpolar amino acid having a saturated or unsaturated straight chain, branched or cyclic hydrocarbon side chain. Examples of genetically encoded aliphatic amino acids include Ala, Leu, Val and Ile. Examples of non-encoded aliphatic amino acids include Nle.

"Polar Amino Acid" refers to a hydrophilic amino acid having a side chain that is charged or uncharged at physiological pH and that has a bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids are generally hydrophilic, meaning that they have an amino acid having a side chain that is attracted by aqueous solution. Examples of genetically encoded polar amino acids include asparagine, glutamine, lysine and serine. Examples of non-genetically encoded polar amino acids include citrulline, homocysteine, N-acetyl lysine and methionine sulfoxide.

"Acidic Amino Acid" refers to a hydrophilic amino acid having a side chain pK value of less than 7. Acidic amino acids typically have negatively charged side chains at physiological pH due to loss of a hydrogen ion. Examples

of genetically encoded acidic amino acids include aspartic acid (aspartate) and glutamic acid (glutamate).

"Basic Amino Acid" refers to a hydrophilic amino acid having a side chain pK value of greater than 7. Basic amino acids typically have positively charged side chains at physiological pH due to association with hydronium ion. Examples of genetically encoded basic amino acids include arginine, lysine and histidine. Examples of non-genetically encoded basic amino acids include the non-cyclic amino acids ornithine, 2,3-diaminopropionic acid, 2,4-diaminobutyric acid and homoarginine.

"Ionizable Amino Acid" refers to an amino acid that can be charged at a physiological pH. Such ionizable amino acids include acidic and basic amino acids, for example, D-aspartic acid, D-glutamic acid, D-histidine, D-arginine, D-lysine, D-hydroxylysine, D-ornithine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine, L-lysine, L-hydroxylysine or L-ornithine.

As will be appreciated by those having skill in the art, the above classifications are not absolute. Several amino acids exhibit more than one characteristic property, and can therefore be included in more than one category. For example, tyrosine has both a nonpolar aromatic ring and a polar hydroxyl group. Thus, tyrosine has several characteristics that could be described as nonpolar, aromatic and polar. However, the nonpolar ring is dominant and so tyrosine is generally considered to be nonpolar. Similarly, in addition to being able to form disulfide linkages, cysteine also has nonpolar character. Thus, while not strictly classified as a hydrophobic or nonpolar amino acid, in many instances cysteine can be used to confer hydrophobicity or nonpolarity to a peptide.

The classifications of the above-described genetically encoded and non-encoded amino acids are summarized in Table 2, below. It is to be understood that Table 2 is for illustrative purposes only and does not purport to be an exhaustive list of amino acid residues that may comprise the peptides and peptide analogues described herein. Other amino acid residues that are useful for making the peptides described herein can be found, e.g., in Fasman, 1989,

CRC Practical Handbook of Biochemistry and Molecular Biology, CRC Press, Inc., and the references cited therein. Another source of amino acid residues is provided by the website of RSP Amino Acids Analogues, Inc. (www.amino-acids.com). Amino acids not specifically mentioned herein can be conveniently classified into the above-described categories on the basis of known behavior and/or their characteristic chemical and/or physical properties as compared with amino acids specifically identified.

TABLE 2

Classification	**Genetically Encoded**	**Genetically Non-Encoded**
Nonpolar		
Aromatic	F, Y, W	Phg, Nal, Thi, Tic, Phe(4-Cl), Phe(2-F), Phe(3-F), Phe(4-F), Pyridyl Ala, Benzothienyl Ala
Aliphatic	A, V, L, I	t-BuA, t-BuG, MeIle, Nle, MeVal, Cha, bAla, MeGly, Aib
Other Nonpolar	M, G, P	
Polar		
Acidic	D, E	
Basic	H, K, R	Dpr, Orn, hArg, Phe(p-NH_2), DBU, A_2 BU
Neutral Polar	S, T, Y, Q, N, D, E, H, R, K,	Cit, AcLys, MSO, hSer, Orn,
Cysteine-Like	C	Pen, hCys, β-methyl Cys

In some embodiments, polar amino acids contemplated by the present invention include, for example, arginine, asparagine, aspartic acid, glutamic acid, glutamine, histidine, lysine, hydroxylysine, ornithine, serine, threonine, the corresponding β-amino acids, and structurally related amino acids. In one embodiment the polar amino is an ionizable amino acid such as arginine, aspartic acid, glutamic acid, histidine, hydroxylysine, lysine, or ornithine.

Examples of nonpolar or nonpolar amino acid residues that can be utilized include, for example, alanine, valine, leucine, methionine, isoleucine, phenylalanine, tryptophan, tyrosine and the like.

In addition, the amino acid sequence of a peptide can be modified so as to result in a peptide variant that includes the substitution of at least one amino acid residue in the peptide for another amino acid residue, including substitutions that utilize the D rather than L form.

One or more of the residues of the peptide can be exchanged for another, to alter, enhance or preserve the biological activity of the peptide. Such a variant can have, for example, at least about 10% of the biological activity of the corresponding non-variant peptide. Conservative amino acid substitutions are often utilized, i.e., substitutions of amino acids with similar chemical and physical properties, as described above.

Hence, for example, conservative amino acids substitutions involve exchanging aspartic acid for glutamic acid; exchanging lysine for arginine or histidine; exchanging one nonpolar amino acid (alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, valine,) for another; and exchanging one polar amino acid (aspartic acid, asparagine, glutamic acid, glutamine, glycine, serine, threonine, etc.) for another. After the substitutions are introduced, the variants are screened for biological activity.

In one embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula I:

$$\begin{bmatrix} (Y_1)_p\text{-}(X_1)_m\text{-}(Y_2)_p\text{-}(X_2)_p\text{-}(Y_3)_p\text{-}(X_3)_p\text{-}(Y_4)_p\text{-}(X_4)_p\text{-}(Y_5)_p\text{-}(X_5)_p \\ (X_{10})_p\text{-}(Y_{10})_p\text{-}(X_9)_p\text{-}(Y_9)_p\text{-}(X_8)_p\text{-}(Y_8)_p\text{-}(X_7)_p\text{-}(Y_7)_p\text{-}(X_6)_p\text{-}(Y_6)_p \end{bmatrix}$$

I

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid; and

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In another embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula II:

$$\begin{bmatrix} (\text{D-X}_1\text{ -L-X}_2)_m - (\text{D-Y}_1 - \text{L-Y}_2)_p - (\text{L-X}_3 - \text{D-X}_4)_p - (\text{L-Y}_3 - \text{D-Y}_4)_p \\ (\text{L-Y}_8 - \text{D-Y}_7)_p - (\text{D-X}_8\text{ -L-X}_7)_p - (\text{D-Y}_6 - \text{L-Y}_5)_p - (\text{L-X}_6 - \text{D-X}_5)_p \end{bmatrix}$$

II

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, and Y_8 is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In yet another embodiment, the cyclic peptides of the invention can have an amino acid sequence having formula III:

$$\begin{bmatrix} (X_1)_p\text{-}(X_2)_p\text{-}(X_3)_p\text{-}(X_4)_m\text{-}(X_5)_p\text{-}(X_6)_p\text{-}(X_7)_p\text{-}(X_8)_p\text{-}(X_9)_p\text{-}(X_{10})_p \\ (Y_{10})_p\text{-}(Y_9)_p\text{-}(Y_8)_p\text{-}(Y_7)_p\text{-}(Y_6)_p\text{-}(Y_5)_p\text{-}(Y_4)_p\text{-}(Y_3)_p\text{-}(Y_2)_p\text{-}(Y_1)_p \end{bmatrix}$$

III

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In yet another embodiment, the cyclic peptide has an amino acid sequence of formula IVa or IVb:

$$D\text{-}X_1 - (L\text{-}X_2 - D\text{-}X_3)_n - (L\text{-}Y_1 - D\text{-}Y_2)_m - L\text{-}Y_3 \qquad \text{IVa}$$

or

$$L\text{-}X_1 - (D\text{-}X_2 - L\text{-}X_3)_n - (D\text{-}Y_1 - L\text{-}Y_2)_m - D\text{-}Y_3 \qquad \text{IVb}$$

wherein:

n is an integer ranging from 0 to 4;

m is an integer ranging from 1 to 7;

X_1, X_2 and X_3 are each a separate a polar amino acid;

Y_1, Y_2 and Y_3 are each a separate nonpolar amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

In another embodiment, the cyclic peptide has an amino acid sequence of formula Va or Va:

$$D\text{-}X_1\text{-}L\text{-}X_2 - (D\text{-}Y_1 - L\text{-}Y_2)_q \qquad \text{Va}$$

or

$$L\text{-}X_1 - D\text{-}X_2 - (L\text{-}Y_1 - D\text{-}Y_2)_q \qquad \text{Vb}$$

wherein:

q is an integer ranging from 2 to 7;

X_1 and X_2 are separately polar amino acids;

Y_1 and Y_2 are separately nonpolar amino acids.

For example, the X amino acids in the above formulae can be D-serine, D-threonine, D-asparagine, D-glutamine, D-aspartic acid, D-glutamic acid, D-

histidine, D-arginine, D-lysine, D-hydroxylysine, D-ornithine, L-serine, L-threonine, L-asparagine, L-glutamine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine, L-lysine, L-hydroxylysine or L-ornithine, provided that the α-cyclic peptide has a sequence of alternating D- and L-α-amino acids.

In some embodiments, one or more of the X amino acids are ionizable amino acids. Such ionizable amino acids include, for example, D-aspartic acid, D-glutamic acid, D-histidine, D-arginine, D-lysine, D-hydroxylysine, D-ornithine, L-aspartic acid, L-glutamic acid, L-histidine, L-arginine, L-lysine, L-hydroxylysine or L-ornithine.

The Y amino acids in the above formulae can be, for example, L-alanine, L-valine, L-leucine, L-methionine, L-isoleucine, L-phenylalanine, L-tryptophan, L-tyrosine, D-alanine, D-valine, D-leucine, D-methionine, D-isoleucine, D-phenylalanine, D-tyrosine or D-tryptophan, provided that the α-cyclic peptide has a sequence of alternating D- and L-α-amino acids. In other embodiments, the Y amino acids may be L-tryptophan, D-tryptophan, L-leucine or D-leucine, provided that the cyclic peptide has a sequence of alternating D- and L-amino acids.

The cyclic peptides of the present invention, for example, include any of SEQ ID NO:5, 7-22, 26-29, 40, 41, 43-55, 57, 58, 61-67, 72-77, 79-89, 91-93, 97-102, 107, 109-112, 114-117, 119-122, 125, 126, 128, 129, 133, 139, 140 or 141. In some embodiments the cyclic peptides employed are those with SEQ ID NO:8, 9, 12, 17, 18, 26, 29, 47-52, 61, 63, 67, 68, 72-77, 84, 85, 87-89, 91-93, 100, 102, 107, 111, 112, 119, 125 and 139. Formulations or compositions containing the present cyclic peptides can include a mixture of two or more cyclic peptides.

The present isolated, purified peptides or variants thereof, can be synthesized *in vitro*, e.g., by the solid phase peptide synthetic method or by enzyme catalyzed peptide synthesis or with the aid of recombinant DNA technology. Solid phase peptide synthetic method is an established and widely used method, which is described in references such as the following: Stewart et al., Solid Phase Peptide Synthesis, W. H. Freeman Co., San Francisco (1969); Merrifield, J. Am. Chem. Soc. *85* 2149 (1963); Meienhofer in "Hormonal

Proteins and Peptides," ed.; C.H. Li, Vol.2 (Academic Press, 1973), pp.48-267; and Bavaay and Merrifield, "The Peptides," eds. E. Gross and F. Meienhofer, Vol.2 (Academic Press, 1980) pp.3-285. These peptides can be further purified by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on an anion-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; ligand affinity chromatography; or crystallization or precipitation from non-polar solvent or nonpolar/polar solvent mixtures. Purification by crystallization or precipitation is preferred.

To identify highly active cyclic peptides that have little or no undesired toxicity for mammalian cells, individual cyclic peptides, or libraries of cyclic peptides can be made and the individual cyclic peptides or cyclic peptides from those libraries can be screened for anti-cancer activity and toxicity using assays and techniques known in the art. For example, libraries of peptides can be made using a one-bead-one-compound strategy provided by Lam et al. (97 Chem. Rev. 411-448 (1997) or synthesized on macrobeads by a split and pool method of Furka, et al. (37 Int. J. Pept. Prot. Res. 487-493(1991)). Mass spectrometric sequence analysis techniques enable rapid identification of every peptide within a given library. *See,* Biemann, K. 193 Methods Enzymol. 455 (1990). In general, synthetic operations, including peptide cyclization, are performed on solid support to avoid laborious and difficult to automate solution-phase operations. Moreover, the final product of the synthesis regimen is generally sufficiently pure for biological assays without laborious purification procedures. Peptide yields from each synthesis can be sufficient for performing 50 to 100 assays. Rapid, automatic mass-spectrometry-based peptide sequence analysis can be performed to identify peptide sequences that have high activity and to discard peptide sequences with low activity.

The synthetic approach employed can provide individually separable and identifiable peptide sequences to avoid the use of combinatorial library mixtures and laborious deconvolution techniques. However, libraries of impure mixtures

of peptides can also be generated for testing. Impure preparations of peptides can be used for quick screening of combinations of sequences. When a mixture of peptides shows activity, the peptides in the mixture can either be individually isolated and tested or pure peptides having sequences known to be present in the impure mixture can be individually prepared and tested.

Salts of carboxyl groups of a peptide or peptide variant of the invention may be prepared in the usual manner by contacting the peptide with one or more equivalents of a desired base such as, for example, a metallic hydroxide base, e.g., sodium hydroxide; a metal carbonate or bicarbonate base such as, for example, sodium carbonate or sodium bicarbonate; or an amine base such as, for example, triethylamine, triethanolamine, and the like.

N-acyl derivatives of an amino group of the peptide or peptide variants may be prepared by utilizing an N-acyl protected amino acid for the final condensation, or by acylating a protected or unprotected peptide. O-acyl derivatives may be prepared, for example, by acylation of a free hydroxy peptide or peptide resin. Either acylation may be carried out using standard acylating reagents such as acyl halides, anhydrides, acyl imidazoles, and the like. Both N-acylation and O-acylation may be carried out together, if desired.

Acid addition salts of the peptide or variant peptide, or of amino residues of the peptide or variant peptide, may be prepared by contacting the peptide or amine with one or more equivalents of the desired inorganic or organic acid, such as, for example, hydrochloric acid. Esters of carboxyl groups of the peptides may also be prepared by any of the usual methods known in the art.

The invention also contemplates cyclic peptides composed of one or more β amino acids. Such β-amino acids can be substituted along their peptide backbones by one to two substituents. Such substituents can include cycloalkyl, cycloalkenyl, and heterocyclic rings that encompass the α and β carbons of the β-peptide backbone. These rings can be, for example, C_3 -C_8 cycloalkyl, cycloalkenyl or heterocyclic rings having one or more nitrogen atoms as the sole heteroatom, and can be substituted or unsubstituted. The substituents on the ring or on the α and β carbons of the β-peptide can be, for example, hydroxy, linear

or branched C_1-C_6-alkyl, alkenyl, alkynyl; hydroxy-C_1-C_6-alkyl; amino-C_1-C_6-alkyl; C_1-C_6 -alkyloxy, C_1-C_6-alkoxy-alkyl; C_1-C_6 - amino; mono- or di-C_1-C_6-alkylamino; carboxamido; carboxamido-C_1-C_6-alkyl; sulfonamido; sulfonamido-C_1-C_6-alkyl, urea, cyano, fluoro, thio; C_1-C_6 -alkylthio; mono- or bicyclic aryl; mono- or bicyclic heteroaryl having up to 5 heteroatoms selected from N, O, and S; mono- or bicyclic aryl- C_1-C_6 and heteroaryl -C_1-C_6 -alkyl and the like.

In one embodiment, the cyclic β-peptides of the invention can have an amino acid sequence of formula VI:

$$\left[(Z_1)_p\text{-}(Z_2)_p\text{-}(Z_3)_p\text{-}(Z_4)_p\text{-}(Z_5)_p\text{-}(Z_6)_p\text{-}(Z_7)_p\text{-}(Z_8)_p\text{-}(Z_9)_p\text{-}(Z_{10})_p \; (Z_{20})_p\text{-}(Z_{19})_p\text{-}(Z_{18})_p\text{-}(Z_{17})_p\text{-}(Z_{16})_p\text{-}(Z_{15})_p\text{-}(Z_{14})_p\text{-}(Z_{13})_p\text{-}(Z_{12})_p\text{-}(Z_{11})_p \right]$$

VI

wherein:

each p is separately an integer ranging from 0 to 7;

each $Z_1, Z_3, Z_5, Z_7, Z_9, Z_{11}, Z_{13}, Z_{15}, Z_{17}$, and Z_{19} is separately a monosubstituted β-amino acid;

each $Z_2, Z_4, Z_6, Z_8, Z_{10}, Z_{12}, Z_{14}, Z_{16}, Z_{18}$, and Z_{20} is separately a disubstituted β-amino acid; and

wherein the cyclic β-peptide has a sequence of from three to about ten homochiral β-amino acids.

Supramolecular Structures

While not intending to be bound by any particular theory or mechanism, according to the present invention the cyclic peptides provided herein are believed self-assemble into supramolecular structures. Self-assembly means that a collection of cyclic peptides can associate to form a supramolecular structure on or within a cellular membrane without the assistance of anything other than the components of the cellular membrane. In general, the physical and chemical properties of the cellular membrane may facilitate self-assembly of the cyclic peptides and the interaction between the components of cancer cellular

membranes and the cyclic peptides may determine whether the cyclic peptides are selective for those cellular membranes.

Formation of supramolecular structures by the peptides of the invention is supported by high-resolution imaging using cryo-electron microscopy, electron diffraction, Fourier-transform infrared spectroscopy, and molecular modeling. Supramolecular structures have been further characterized by IR spectroscopy, low-dose electron microscopy, and the analysis of electron diffraction patterns.

According to the present invention, cyclic peptide structures that are made up of an even number of alternating D- and L-amino acid residues are believed to adopt or sample a flat ring-shaped conformation in which all backbone amide functionalities lie approximately perpendicular to the plane of the ring structure. Similarly, cyclic peptides made up of β-amino acids can also adopt a flat-ring structure. In this flat-ring conformation, while not intending to be bound by any particular theory or mechanism of action, it is believed that the peptide subunits can stack, under favorable conditions, to furnish a contiguous hydrogen bonded hollow tubular structure that is referred to herein as a nanotube (see Figures 1 and 5).

For example, controlled acidification of alkaline solutions of peptide SEQ ID NO:1 (cyclo[-(Gln-D-Ala-Glu D-Ala)$_3$-]) yielded rod shaped crystalline materials that appeared under transmission electron microscopy as organized bundles of tightly packed tubular structures. Low dose cryo microscopy, according to the method of M. Adrian et al. (308 *Nature* 32-36 (1984)) and of R.A. Milligan et al. (13 *Ultramicroscopy* 1-10 (1984)) revealed longitudinal striations with spacing of approximately 25 Å as expected for the center to center spacing for closely packed tubular structures. Electron diffraction patterns display axial spacing of 4.80 Å that is in agreement with the peptide stacking and the formation of tight network of hydrogen bonded b-sheet type structure. The meridonial spacing in the electron diffraction patterns display spacing of 12.67±0.06 Å and 21.94±0.05 Å characteristic of a hexagonal body centered packing of nanotubes. Hexagonal lattice resulting from the close packing of

cylinders of radius *r* displays the characteristic two principle lattice planes of radius *r* and *r* such as the one observed here (*r*=12.67 Å and *r* =21.94 Å). The periodicity in this packing produces diffraction spots at 1/*r*, 2/*r*, and so on, and at 1/*r*, and 2/*r*, and so on. The observed electron diffraction patterns on the meridional axes extend to third order reflections (4.1 Å) signifying the ordered and crystalline state of nanotube structures. The diffraction patterns also showed a unit cell with an angle of 99° and no other symmetry than the center of symmetry pursuant to Friedel's law.

A three-dimensional supramolecular structure model was built using the parameters obtained from the electron diffraction patterns—unit cell with *a*=9.6 Å (2x4.80 Å for the antiparallel dimer), *b*=*c*=25.66 Å (2x12.67÷*Cos*9), α=120°, and β=γ=99°. The model shows structure factors similar to the patterns observed in the electron diffraction thus supporting the proposed three-dimensional model. Involvement of intermolecular hydrogen bonding network in a tube-like assembly is also supported by FT-IR spectroscopic analysis according to the method of S. Krimm et al. (*Advances in Protein Chemistry*; Anfinsen, C. B., Edsall, J. T.; Richards, F. M. Eds.; Academic Press: Orlando, 1986, pages 181-364). Nanotubes display characteristic IR features of a β-sheet structure signified not only by the *amide I* bands at 1626 cm^{-1} and 1674 cm^{-1} and an *amide II* band at 1526 cm^{-1}, but also by the observed NH stretching frequency at 3291 cm^{-1} supporting formation of a tight network of hydrogen bonds.

The IR spectrum is similar to tubular structures that have been discovered in nature. For example, nanotubular structures for some of the present peptides can be conceptually related to the structure of crystalline linear Gramicidin A that is known to form dimeric β-helical structures. Gramicidin A has *amide I* bands at 1630, 1685 cm^{-1}, an *amide II* band at 1539 cm^{-1}, and an NH stretching frequency at 3285 cm^{-1}. (V.M. Naik et al. in *Biophys. J.* (1986), vol. *49*, pages 1147-1154.) The observed frequency of NH stretching mode correlates to an average intersubunit distance of 4.76 Å that is in close agreement with the value of 4.80 Å obtained independently from the electron diffraction patterns.

The pore size, or internal diameter, of self-assembled nanotubes can be adjusted by the ring size of the peptide subunit employed. A twelve-residue cyclic peptide structure, for example cyclo[-(Gln-D-Ala-Glu-D-Ala)$_3$-] (SEQ ID NO:1), has a diameter of about 13 Å. The eight residue cyclic peptide cyclo[-(Trp-D-Leu)$_3$-Gln-D-Leu-] (SEQ ID NO:2) has a diameter of approximately 7.5 Å in diameter. A cyclic peptide having SEQ ID NO:2 in synthetic phosphatidylcholine liposomes displays an FTIR *amide-I* band at 1624 cm^{-1} and an observed N-H stretching frequency at 3272 cm^{-1} that support formation of a tight network of β-sheet-like hydrogen bonds with an average intersubunit distance of 4.7 Å to 4.8 Å.

The flat, ring-shaped cyclic peptides of the present invention are not only structurally predisposed toward intermolecular interaction, but are also energetically favored to self-assemble on selected cancer cell membranes and permeabilize cancer cells through formation of pores or other membrane destabilizing structures.

Formation of supramolecular structures that can permeabilize membranes was also inferred from proton transport activity. Vesicles were prepared having pH 6.5 inside and pH 5.5 in the outside bulk solution. The collapse of the imposed pH gradient in these vesicles, upon formation of the putative transmembrane channel structure, was studied by monitoring the fluorescence intensity of an entrapped pH-sensitive dye. (V.E. Carmichael et al, in *J. Am. Chem. Soc.* (1989), vol. 111, pages 767-769). Addition of peptide cyclo[-(Trp-D-Leu)$_3$-Gln-D-Leu-] (SEQ ID NO:2) to such vesicles suspensions causes a rapid collapse of the pH gradient.

Unilamellar vesicles were prepared by the reverse-phase evaporation using DPPC, OPPC, cholesterol in the ratio of 1:1:2 in a solution containing 5(6)-carboxyfluorescein (20 mM in phosphate/saline buffer: 137 mM NaCl, 2.6 mM KCl, 6.4 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 6.5) according to the method of F. Szoka et al. in *Proc. Natl. Acad. Sci. USA* (1978), vol. 75, pages 4194-4198. Liposomes were then sized by multiple extrusions through Nucleopore® polycarbonate membranes (10 times, 50 psi, using 0.8 and 2x0.4 micron filter

stacks) and the untrapped 5(6)-carboxyfluorescein was removed by size exclusion chromatography (Sephadex G-25 column 1x30 cm) using the same phosphate/saline buffer according to the method of F. Olson et al. in *Biochim. Biophys. Acta* (1979), vol. 557, pages 9-23. Vesicles formed in this way are approximately 150 nanometers in diameter as determined by electron microscopy. (R.R.C. New, Ed. *Liposomes*, Oxford University Press, 1990). In each experiment, 70 ml of the stock vesicle solution ($3.5x10^{-3}$ M in phospholipids) was added to pH 5.5 buffer (1.3 ml, 137 mM NaCl, 2.6 mM KCl, 6.4 Na_2HPO_4, 1.4 KH_2PO_4) and placed in a 1 cm quartz cuvette inside a stirring thermojacketed sample holder of the fluorescence instrument and equilibrated at 25 °C for 15 minutes with gentle stirring. To the cuvette, through an injector port, 25 ml of the peptide dissolved in DMSO was added with continuous fluorescence monitoring at 520 nm (excitation at 470 nm). The observed data were then normalized for comparison into the fractional change in fluorescence $((I_0-I_t)/(I_0-I_\infty))$ (V.E. Carmichael et al, in *J. Am. Chem. Soc.* (1989), vol. 111, pages 767-769)).

Control studies, monitoring the release of carboxyfluorescein dye entrapped in liposomes, indicated that the collapse of the pH gradient was not due to the rupturing of the liposomes nor due to the small amounts of organic solvents (<2% DMSO) employed in these studies. Furthermore, the control peptide cyclo[-(Gln-D-Leu)$_4$] that lacks the appropriate surface characteristics for partitioning into the liposomes, does not display any ion transport activity under similar conditions. A second control peptide *cyclo*[-(^{Me}N-D-Ala-Phe)$_4$-] that has the desirable hydrophobic surface characteristics but lacks the propensity for participating in extended hydrogen bonding network, was also designed and tested for ion transport activity. The ring structure of this peptide is N-methylated on one face. Such N-methylation does not adversely affect the ability of the peptide to interact with liposomal membranes but predisposes peptides toward a dimeric cylindrical structure that cannot span a normal liposomal membrane. Thus, although the peptide has been shown to partition effectively into liposomes, it does not promote proton transport activity in the

above vesicle experiments. Together, these experiments indicate that not only are the side chains displayed on cyclic peptides important for membrane interaction, but also the peptide backbone can participate in extended intermolecular hydrogen bonding, for example, to facilitate membrane permeablization.

Methods of Use

The present invention is directed to methods of treating cancer in an animal, for example, for human and veterinary uses, which include administering to a subject animal (*e.g.*, a human), a therapeutically effective amount of a cyclic peptide of the present invention. While not intending to be bound by any particular theory or mechanism of action, according to the present invention, it is believed that the cyclic peptide undergoes self-assembly to form a supramolecular structure that causes cancer cellular membrane permeation, destabilization or depolarization.

Treatment of, or treating, cancer is intended to include the alleviation of or diminishment of at least one symptom typically associated with the disease. The treatment also includes alleviation or diminishment of more than one symptom. The treatment may cure the cancer, *e.g.*, it may substantially kill the cancer cells and/or it may arrest or inhibit the growth of the cancerous tumor.

Cancers that can be treated by the present cyclic peptides include solid mammalian tumors as well as hematological malignancies. Solid mammalian tumors include cancers of the head and neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin central nervous system; sarcomas of the soft tissue and bone; and melanoma of cutaneous and intraocular origin. Hematological malignancies include childhood leukemia and lymphomas, Hodgkin's disease, lymphomas of lymphocytic and cutaneous origin, acute and chronic leukemia, plasma cell neoplasm and cancers associated with AIDS. In addition, a cancer at any stage of progression can be treated, such as primary, metastatic, and recurrent cancers. Information regarding numerous

types of cancer can be found, e.g., from the American Cancer Society (www.cancer.org), or from, e.g., Wilson et al. (1991) Harrison's Principles of Internal Medicine, 12.sup.th Edition, McGraw-Hill, Inc. Both human and veterinary uses are contemplated.

Anti-cancer activity can be evaluated against varieties of cancers using methods available to one of skill in the art. Anti-cancer activity, for example, is determined by identifying the lethal dose (LD_{100}) or the 50% effective dose (ED_{50}) or the dose that inhibits growth at 50% (GI_{50}) of a cyclic peptide of the present invention that prevents the growth of a cancer. In one aspect, anti-cancer activity is the amount of the peptide that kills 50% or 100% of the cancer cells, for example, when measured using standard dose response methods.

The present invention also provides a method of evaluating a therapeutically effective dosage for treating a cancer with a cyclic peptide having an amino acid sequence of alternating D- and L-amino acids, or of three to about ten homochiral β-amino acids, that includes determining the LD_{100} or ED_{50} of the cyclic peptide *in vitro*. Such a method permits calculation of the approximate amount of cyclic peptide needed per volume to inhibit cancer cell growth or to kill 50% to 100% of the cancer cells. Such amounts can be determined, for example, by standard microdilution methods.

According to the present invention, the cyclic peptides provided herein do not have substantial or undesired toxicity against normal mammalian or other animal cells. While not intending to be bound by any particular theory or mechanism of action, hemolysis is one way to measure whether a cyclic peptide can self-assemble within normal mammalian or other animal cell membranes. If a cyclic peptide can self-assemble within a membrane, the membrane will tend to become depolarized and permeabilized. Red blood cells are conveniently used to test for membrane depolarization and permeabilization, because they undergo hemolysis, which can be detected as the release of hemoglobin from the cell. Hemolysis can be observed by methods available to one of skill in the art. For example, after exposure to the present cyclic peptides, the release of hemoglobin can be observed spectrophotometrically by observing the

absorbance of light at wavelengths characteristic of hemoglobin, for example, at 543 nm. Control samples can be used, for example, the medium in which the cells are tested or maintained can serve as a zero blank. A second control can be used to determine the absorbance value for 100% hemolysis. Such a second control can be a sample that is identical to the test animal cell sample but which has been sonicated to completely disrupt the cells.

Screening Methods

Assays may be used to identify cyclic peptides that can selectively interact with a cancer cell of interest. A wide variety of assays may be used for this purpose. See, for example, the assays carried out within the National Cancer Institute's "In Vitro Cell Line Screening Project." In general, such an assay can involve contacting a cancer cell of interest with at least one cyclic peptide and observing whether the cyclic peptide kills the cancer cell and/or has other deleterious effects upon that cell.

Methods available in the art can also be used for determining whether the cyclic peptides of the invention interact with the membrane of a cancer cell of interest. For example, cyclic peptides can be labeled with a reporter molecule that permits detection of the peptide. After labeling, the cyclic peptides can be contacted with the cancer cell of interest for a time and under conditions that permit binding or association of the peptide to cellular membranes. The cells can be washed with physiological solutions to remove unbound or unassociated cyclic peptides, and the cells can then be observed to ascertain whether the reporter molecule is bound or associated with the cells or the cellular membranes. In another embodiment, one of skill in the art can test whether the cyclic peptide(s) can selectively penetrate the membranes of selected cancer cells. This may be done by examining whether the reporter molecule remains associated with the cellular membranes of the cancer cell or whether the reporter molecule becomes associated with the interior of the cell.

Reporter molecules that can be employed include any detectable compound or molecule available to one of skill in the art that is conjugated directly or indirectly to a cyclic peptide of the invention. The label may itself be

detectable (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable.

Deleterious effects upon the cancer cell of interest can also be detected as an indication of an interaction between a cyclic peptide of the invention and the cell. Such deleterious effects can involve any evidence that the cyclic peptide has had an adverse or cytotoxic effect upon the cell. For example, one of skill in the art can test whether the cyclic peptide(s) kill the cancer cell, cause membrane depolarization, cause permeabilization of the membranes of the cell, or tend to lyse the cancer cells.

Of particular interest are screening assays for cyclic peptides that have little interaction with, and low toxicity for, normal human or other animal cells but that have good anti-cancer properties (depolarizing or permeabilizing cancer cell membranes, lysing or otherwise killing the cancer cell).

Generally, pluralities of assays are performed in parallel with different cyclic peptides at different concentrations to obtain a differential response to the various concentrations. Typically, at least one control assay is included in the testing. Such a control can be a negative control involving exposure of the cancer cells of interest to a physiologic solution containing no cyclic peptide. Another control can involve exposure of the cancer cell of interest to a cyclic peptide that has already been observed to adversely affect the cancer cell of interest, or a second cell that is related to the cell of interest. Another control can involve exposing a cell of interest to a known therapeutic agent that has a desired affect on the cancer cell of interest, for example, an anti-cancer agent with known efficacy at a particular concentration or dosage. One of skill in the art can readily select control compounds and conditions that facilitate screening and analysis of the effects of the cyclic peptides on a cancer cell of interest.

Candidate cyclic peptides are obtained from a wide variety of sources including libraries of cyclic peptides generated as described herein. Cyclic peptides can also be rationally designed and synthesized to have specific structural features selected by one of skill in the art.

Any cell type available to one of skill in the art can be assayed by these methods. For example, any mammalian or other animal cancer cell type can be screened to assess whether the cyclic peptides of the invention can selectively interact therewith. Mammalian or other animal cells can also be screened to ascertain whether the peptides of the invention selectively interact therewith and/or to determine whether the peptides of the invention do not interact, bind, lyse, kill or otherwise adversely affect the viability of the mammalian or other animal cell.

In one aspect, mammalian or other animal red blood cells are screened with the cyclic peptides to ascertain whether the cyclic peptides have an adverse effect on the red blood cells. As is known to one of skill in the art, the membrane of red blood cells tends to be more susceptible to lysis than many other mammalian or other animal cell types. Hence, while not intending to be bound by any particular theory or mechanism of action, red blood cells are a useful cell type for quickly screening whether a cyclic peptide would be expected to have any adverse effects on other mammalian or other animal cell types and/or *in vivo* after therapeutic administration. Methods of assaying for cell lysis are available in the art. For example, using procedures described herein, red blood cells can be tested to ascertain whether hemolysis has occurred upon exposure to at least one cyclic peptide of the invention. When it is established that a cyclic peptide causes little hemolysis of red blood cells, the peptide is tested against other mammalian or other animal cell types or used for in vivo testing in standard animal models.

Conditions for screening cyclic peptides include conditions that are used by one of skill in the art to grow, maintain or otherwise culture cell types of interest. Cancer cell types of interest should be assayed under conditions where they would be healthy but for the presence of the cyclic peptide(s). Controls can be performed where the cell types are maintained under the selected culture conditions and not exposed to a cyclic peptide, to assess whether the culture conditions influenced the viability of the cells. One of skill in the art can also perform the assay on cells that have been washed in simple physiological

solutions, such as buffered saline, to eliminate, or test for, any interaction between the cyclic peptides or cells and the components in the culture media. However, culture conditions for the assays generally include providing the cells with the appropriate concentration of nutrients, physiological salts, buffers and other components typically used to culture or maintain cells of the selected type. A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, albumin, and serum (e.g. fetal calf serum) that are used to mimic the physiologic state of the cell types of interest. Conditions and media for culturing, growing and maintaining cells are available to one of skill in the art.

The selected reagents and components are added to the assay in the order selected by one of skill in the art. In general, the cyclic peptides are added last to start the assay. Assays are performed at any suitable temperature, typically between 4 °C and 40° C. For example, the temperature may generally range from about room temperature (about 20 °C) to about 37°C. Incubation periods are selected to ascertain the optimal range of activity or to insure that the cyclic peptides do not adversely affect the cell type of interest. However, incubation times can be optimized to facilitate rapid high-throughput screening. Typically incubation times are between about one minute and about five days, such as a range from about 30 minutes to about 3 days.

Cyclic peptides having the desired selectivity and activity *in vitro* may be tested for activity and/or lack of toxicity *in vivo*, in an appropriate animal model. Such animal models include primates as well as mice, rats, rabbits, cats, dogs, pigs, goats, cattle or horses. For example, the mouse is a convenient animal model for testing whether cyclic peptides of the invention have toxic effects and/or to determine whether the cyclic peptides can inhibit the growth of a cancer cell.

One of skill in the art can readily perform *in vivo* evaluation of the cyclic peptides of the invention. For toxicity testing, a series of cyclic peptides at different test dosages can be separately administered to different animals. A single dose or, a series of dosages can be administered to the animal. A test

period is selected that permits assessment of the effects of the peptide(s) on the animal. Such a test period can run from about one day to about several weeks or months.

The effect of a cyclic peptide(s) on an animal can be determined by observing whether the peptide adversely affects the behavior (e.g., lethargy, hyperactivity) and physiological state of the animal over the course of test period. The physiological state of the animal can be assessed by standard procedures. For example, during the test period one of skill in the art can draw blood and collect other bodily fluids to test, for example, for various enzymes, proteins, metabolites, and the like. One of skill in the art can also observe whether the animal has bloating, loss of appetite, diarrhea, vomiting, blood in the urine, loss of consciousness, and a variety of other physiological problems. After the test period, the animal can be sacrificed and anatomical, pathological, histological and other studies can be performed on the tissues or organs of the animal.

In general, to determine whether one or more cyclic peptides of the invention can inhibit cancer cell growth, mice are infected with the selected cancer and a selected test dosage of one or more cyclic peptides is administered shortly thereafter. Mice are observed over the course of several days to several weeks to ascertain whether the cyclic peptide protects the mice from the cancer. At the end of the test period, mice can be sacrificed and examined to ascertain whether the cyclic peptide has optimally protected the mice from cancer and/or to determine whether any adverse side effects have occurred.

Controls are used to establish the effects of the cancer when the cyclic peptide is not administered. Other controls can also be performed, for example, the safety and efficacy of the present cyclic peptides can be compared to that of known anti-cancer agents. The invention further provides a method of discovering a cyclic peptide capable of selective association with a target biomolecule on a selected cell surface. This method can involve contacting a solution of cyclic peptides with the target biomolecule and determining whether

the peptides spontaneously assemble into a supramolecular structure that selectively associates with the biomolecule.

The target biomolecule can be displayed, for example, on the surface of a living cell or on the surface of a liposome. Alternatively, the peptide can be contacted with the target biomolecule in a hydrogen bond-promoting solution, such as an aqueous solution at a pH of about 7.0 to about 7.6 where simple salts may be present in physiological concentrations.

The method can further include determining the structure of the peptides that spontaneously assemble into the supramolecular structure that selectively associates with the biomolecule.

Cyclic peptides having good anti-cancer properties *in vitro* and/or *in vivo* that also have substantially no toxicity are good candidates for therapeutic development into appropriate dosage forms, as described in more detail below.

Dosages, Formulations and Routes of Administration for the Peptides

The peptides of the invention, including their salts, are administered so as to achieve a reduction in at least one symptom associated with a cancer, tumor, indication or disease, or a decrease in the amount of antibody associated with the cancer, tumor, indication or disease.

To achieve the desired effect(s), the peptide, a variant thereof or a combination thereof, may be administered as single or divided dosages, for example, of at least about 0.01 mg/kg to about 500 to 750 mg/kg, of at least about 0.01 mg/kg to about 300 to 500 mg/kg, at least about 0.1 mg/kg to about 100 to 300 mg/kg or at least about 1 mg/kg to about 50 to 100 mg/kg of body weight, although other dosages may provide beneficial results. The amount administered will vary depending on various factors including, but not limited to, the cyclic peptide chosen, the disease, the weight, the physical condition, the health, the age of the mammal, whether prevention or treatment is to be achieved, and if the peptide is chemically modified. Such factors can be readily determined by the clinician employing animal models or other test systems that are available in the art.

Administration of the therapeutic agents in accordance with the present invention may be in a single dose, in multiple doses, in a continuous or intermittent manner, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the peptides of the invention may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and systemic administration is contemplated.

To prepare the composition, peptides are synthesized or otherwise obtained, purified as necessary or desired and then lyophilized and stabilized. The peptide can then be adjusted to the appropriate concentration, and optionally combined with other agents. The absolute weight of a given peptide included in a unit dose can vary widely. For example, about 0.01 to about 2 g, or about 0.1 to about 500 mg, of at least one peptide of the invention, or a plurality of peptides specific for a particular cell type can be administered. Alternatively, the unit dosage can vary from about 0.01 g to about 50 g, from about 0.01 g to about 35 g, from about 0.1 g to about 25 g, from about 0.5 g to about 12 g, from about 0.5 g to about 8 g, from about 0.5 g to about 4 g, or from about 0.5 g to about 2 g.

Daily doses of the cyclic peptides of the invention can vary as well. Such daily doses can range, for example, from about 0.1 g/day to about 50 g/day, from about 0.1 g/day to about 25 g/day, from about 0.1 g/day to about 12 g/day, from about 0.5 g/day to about 8 g/day, from about 0.5 g/day to about 4 g/day, and from about 0.5 g/day to about 2 g/day.

Thus, one or more suitable unit dosage forms comprising the therapeutic peptides of the invention can be administered by a variety of routes including oral, parenteral (including subcutaneous, intravenous, intramuscular and intraperitoneal), rectal, dermal, transdermal, intrathoracic, intrapulmonary and intranasal (respiratory) routes. The therapeutic peptides may also be formulated for sustained release (for example, using microencapsulation, see WO 94/ 07529, and U.S. Patent No.4,962,091). The formulations may, where appropriate, be

conveniently presented in discrete unit dosage forms and may be prepared by any of the methods well known to the pharmaceutical arts. Such methods may include the step of mixing the therapeutic agent with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into the desired delivery system.

When the therapeutic peptides of the invention are prepared for oral administration, they are generally combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation, or unit dosage form. For oral administration, the peptides may be present as a powder, a granular formulation, a solution, a suspension, an emulsion or in a natural or synthetic polymer or resin for ingestion of the active ingredients from a chewing gum. The active peptides may also be presented as a bolus, electuary or paste. Orally administered therapeutic peptides of the invention can also be formulated for sustained release, e.g., the peptides can be coated, micro-encapsulated, or otherwise placed within a sustained delivery device. The total active ingredients in such formulations comprise from 0.1 to 99.9% by weight of the formulation.

By "pharmaceutically acceptable" it is meant a carrier, diluent, excipient, and/or salt that is compatible with the other ingredients of the formulation, and not deleterious or unsuitably harmful to the recipient thereof.

Pharmaceutical formulations containing the therapeutic peptides of the invention can be prepared by procedures known in the art using well-known and readily available ingredients. For example, the peptide can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, solutions, suspensions, powders, aerosols and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include buffers, as well as fillers and extenders such as starch, cellulose, sugars, mannitol, and silicic derivatives. Binding agents can also be included such as carboxymethyl cellulose, hydroxymethylcellulose, hydroxypropyl methylcellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone. Moisturizing agents can be included such as glycerol, disintegrating agents such as calcium

carbonate and sodium bicarbonate. Agents for retarding dissolution can also be included such as paraffin. Resorption accelerators such as quaternary ammonium compounds can also be included. Surface active agents such as cetyl alcohol and glycerol monostearate can be included. Adsorptive carriers such as kaolin and bentonite can be added. Lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols can also be included. Preservatives may also be added. The compositions of the invention can also contain thickening agents such as cellulose and/or cellulose derivatives. They may also contain gums such as xanthan, guar or carbo gum or gum arabic, or alternatively polyethylene glycols, bentones and montmorillonites, and the like.

For example, tablets or caplets containing the cyclic peptides of the invention can include buffering agents such as calcium carbonate, magnesium oxide and magnesium carbonate. Caplets and tablets can also include inactive ingredients such as cellulose, pre-gelatinized starch, silicon dioxide, hydroxy propyl methyl cellulose, magnesium stearate, microcrystalline cellulose, starch, talc, titanium dioxide, benzoic acid, citric acid, corn starch, mineral oil, polypropylene glycol, sodium phosphate, zinc stearate, and the like. Hard or soft gelatin capsules containing at least one cyclic peptide of the invention can contain inactive ingredients such as gelatin, microcrystalline cellulose, sodium lauryl sulfate, starch, talc, and titanium dioxide, and the like, as well as liquid vehicles such as polyethylene glycols (PEGs) and vegetable oil. Moreover, enteric-coated caplets or tablets containing one or more peptides of the invention are designed to resist disintegration in the stomach and dissolve in the more neutral to alkaline environment of the duodenum.

The therapeutic peptides of the invention can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous, intraperitoneal or intravenous routes. The pharmaceutical formulations of the therapeutic peptides of the invention can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an emulsion or suspension or salve.

Thus, the therapeutic peptides may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion containers or in multi-dose containers. As noted above, preservatives can be added to help maintain the shelve life of the dosage form. The active peptides and other ingredients may form suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active peptides and other ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

These formulations can contain pharmaceutically acceptable carriers, vehicles and adjuvants that are well known in the art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint, chosen, in addition to water, from solvents such as acetone, ethanol, isopropyl alcohol, glycol ethers such as the products sold under the name "Dowanol," polyglycols and polyethylene glycols, C_1-C_4 alkyl esters of short-chain acids, ethyl or isopropyl lactate, fatty acid triglycerides such as the products marketed under the name "Miglyol," isopropyl myristate, animal, mineral and vegetable oils and polysiloxanes.

It is possible to add, if necessary, an adjuvant chosen from antioxidants, surfactants, other preservatives, film-forming, keratolytic or comedolytic agents, perfumes, flavorings and colorings. Antioxidants such as t-butylhydroquinone, butylated hydroxyanisole, butylated hydroxytoluene and α-tocopherol and its derivatives can be added.

Also contemplated are combination products that include one or more cyclic peptides of the present invention and one or more other anti-cancer agents. For example, a variety of antibiotics can be included in the pharmaceutical compositions of the invention, such as aminoglycosides (e.g., streptomycin, gentamicin, sisomicin, tobramycin and amicacin), ansamycins (e.g. rifamycin), antimycotics (e.g. polyenes and benzofuran derivatives), β-lactams (e.g.

penicillins and cephalosporins), chloramphenical (including thiamphenol and azidamphenicol), linosamides (lincomycin, clindamycin), macrolides (erythromycin, oleandomycin, spiramycin), polymyxins, bacitracins, tyrothycin, capreomycin, vancomycin, tetracyclines (including oxytetracycline, minocycline, doxycycline), phosphomycin and fusidic acid.

Additionally, the peptides are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active peptide, for example, in a particular part of the intestinal or respiratory tract, possibly over a period of time. Coatings, envelopes, and protective matrices may be made, for example, from polymeric substances, such as polylactide-glycolates, liposomes, microemulsions, microparticles, nanoparticles, or waxes. These coatings, envelopes, and protective matrices are useful to coat indwelling devices, e.g., stents, catheters, peritoneal dialysis tubing, draining devices and the like.

For topical administration, the therapeutic agents may be formulated as is known in the art for direct application to a target area. Forms chiefly conditioned for topical application take the form, for example, of creams, milks, gels, dispersion or microemulsions, lotions thickened to a greater or lesser extent, impregnated pads, ointments or sticks, aerosol formulations (e.g., sprays or foams), soaps, detergents, lotions or cakes of soap. Other conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols. Thus, the therapeutic peptides of the invention can be delivered via patches or bandages for dermal administration. Alternatively, the peptide can be formulated to be part of an adhesive polymer, such as polyacrylate or acrylate/vinyl acetate copolymer. For long-term applications it might be desirable to use microporous and/or breathable backing laminates, so hydration or maceration of the skin can be minimized. The backing layer can be any appropriate thickness that will provide the desired protective and support functions. A suitable thickness will generally be from about 10 to about 200 microns.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The active peptides can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or 4,051,842. The percent by weight of a therapeutic agent of the invention present in a topical formulation will depend on various factors, but generally will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-85% by weight.

Drops, such as eye drops or nose drops, may be formulated with one or more of the therapeutic peptides in an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs. Drops can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The therapeutic peptide may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may be formulated as a lozenge further comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the composition of the present invention in a suitable liquid carrier.

The pharmaceutical formulations of the present invention may include, as optional ingredients, pharmaceutically acceptable carriers, diluents, solubilizing or emulsifying agents, and salts of the type that are available in the art. Examples of such substances include normal saline solutions such as physiologically buffered saline solutions and water. Specific non-limiting examples of the carriers and/or diluents that are useful in the pharmaceutical formulations of the present invention include water and physiologically acceptable buffered saline solutions such as phosphate buffered saline solutions pH 7.0-8.0.

The peptides of the invention can also be administered to the respiratory tract. Thus, the present invention also provides aerosol pharmaceutical formulations and dosage forms for use in the methods of the invention. In general, such dosage forms comprise an amount of at least one of the agents of the invention effective to treat or prevent the clinical symptoms of a specific infection, indication or disease. Any statistically significant attenuation of one or more symptoms of an infection, indication or disease that has been treated pursuant to the method of the present invention is considered to be a treatment of such infection, indication or disease within the scope of the invention.

Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the therapeutic agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatin or blister packs from which the powder may be administered with the aid of an inhalator, insufflator, or a metered-dose inhaler (see, for example, the pressurized metered dose inhaler (MDI) and the dry powder inhaler disclosed in Newman, S. P. in Aerosols and the Lung, Clarke, S. W. and Davia, D. eds., pp. 197-224, Butterworths, London, England, 1984).

Therapeutic peptides of the present invention can also be administered in an aqueous solution when administered in an aerosol or inhaled form. Thus, other aerosol pharmaceutical formulations may comprise, for example, a physiologically acceptable buffered saline solution containing between about 0.1 mg/ml and about 100 mg/ml of one or more of the peptides of the present invention specific for the indication or disease to be treated. Dry aerosol in the form of finely divided solid peptide or nucleic acid particles that are not dissolved or suspended in a liquid are also useful in the practice of the present invention. Peptides of the present invention may be formulated as dusting powders and comprise finely divided particles having an average particle size of between about 1 and 5 μm, alternatively between 2 and 3 μm. Finely divided particles may be prepared by pulverization and screen filtration using techniques

well known in the art. The particles may be administered by inhaling a predetermined quantity of the finely divided material, which can be in the form of a powder. It will be appreciated that the unit content of active ingredient or ingredients contained in an individual aerosol dose of each dosage form need not in itself constitute an effective amount for treating the particular infection, indication or disease since the necessary effective amount can be reached by administration of a plurality of dosage units. Moreover, the effective amount may be achieved using less than the dose in the dosage form, either individually, or in a series of administrations.

For administration to the upper (nasal) or lower respiratory tract by inhalation, the therapeutic peptides of the invention are conveniently delivered from a nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Nebulizers include, but are not limited to, those described in U.S. Patent Nos. 4,624,251; 3,703,173; 3,561,444; and 4,635,627. Aerosol delivery systems of the type disclosed herein are available from numerous commercial sources including Fisons Corporation (Bedford, Mass.), Schering Corp. (Kenilworth, NJ) and American Pharmoseal Co., (Valencia, CA). For intra-nasal administration, the therapeutic agent may also be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

Furthermore, the active ingredients may also be used in combination with other therapeutic agents, for example, pain relievers, anti-inflammatory agents, antihistamines, bronchodilators and the like, whether for the conditions described or some other condition.

The present invention further pertains to a packaged pharmaceutical composition for controlling cancer such as a kit or other container. The kit or container holds a therapeutically effective amount of a pharmaceutical

composition for controlling cancer and instructions for using the pharmaceutical composition for control of a given cancer type. The pharmaceutical composition includes at least one cyclic peptide of the present invention, in a therapeutically effective amount such that cancer is controlled.

The invention is further illustrated by the following non-limiting Examples.

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EXAMPLE 1

Materials and Methods

Solid Phase Peptide Synthesis

Solvents and reagents: Acetonitrile (ACN, optima grade), dichloromethane (DCM, ACS grade), N,N-dimethylformamide (DMF, sequencing grade), diethyl ether (Et_2O, ACS grade), N,N-diisopropylethylamine (DIEA, peptide synthesis grade) were purchased from Fisher and used without further purification. Trifluoroacetic acid (TFA, New Jersey Halocarbon), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Richelieu Biotechnologies), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP, Novabiochem) were used as obtained. Commercially available amino acids and resins were used as obtained from Bachem, Novabiochem or Advanced Chemtech. The side-chain protections were as follows. For Fmoc synthesis: Arg (Pbf), His (Bcc), Lys (Boc), Ser (t-Bu) and Thr (t-Bu). All other chemicals were used as obtained from Aldrich, Acros, Sigma or Fluka.

Peptide synthesis and cyclization: Linear protected peptides were synthesized on polystyrene solid support using a trityl-functionalized resin and cyclized in the solution phase. The first Fmoc-protected amino acid was loaded onto a Cl-trityl-resin via its α-carboxylate group following standard procedures. Briefly, dry Fmoc-amino acid (1.2 eq. with respect to resin loading) was dissolved in DCM (dried over $NaHCO_3$, 20 mL/g of resin) and DIEA (4 eq.). This mixture was added to a fresh commercially available Cl-trityl resin (loading 0.6-1.2 mmol/g) and continuously shaken for 2 hour, followed by sequential washings with DCM (3x20 mL), MeOH/DIEA/DCM (1:2:17, 3x15 mL) and DCM (3x20 mL). Loading of the first amino acid was estimated by removing the Fmoc group with base from a measured amount of resin and then measuring the UV absorption and concentration of the product in a known sample volume. The rest of the amino acids were sequentially introduced following standard Fmoc protocols. Stewart, J. D., Solid Phase Peptide Synthesis 1984. After the last coupling, N-terminal Fmoc group was removed by treatment with 20%

Piperidine/DMF. The peptide containing resin was washed with DMF (3x20 mL), then with DCM (3x20 mL). The side chain protected linear peptide was cleaved from the resin by sequential shaking with several portions (10-20) of TFA/DCM mixture (1/99) collecting the solution into a flask, containing pyridine (2 mL) and MeOH (5 mL). The completion of the cleavage is achieved when the resin changed its color to dark red. Then the resin was washed with DCM (2x10 mL) and MeOH (2x10 mL). These washings were combined with collected TFA cleavage solutions. The solvent was evaporated under vacuum (1 mm Hg) and the purity of the linear peptide was assessed by MALDI-MS and HPLC. In most cases, purity of the crude linear peptide allowed cyclization without prior purification. Otherwise, linear peptides were purified by reverse phase high performance liquid chromatography (RP-HPLC/C4 or C18, 0.1% TFA in ACN/H_2O).

Cyclization was performed in DMF at a peptide concentration of 1-5 mM using a mixture of PyBOP (5 eq. with respect to crude peptide) and DIEA (40 eq.). The amount of DIEA was adjusted to achieve an apparent pH 9-10, which was assessed by applying a drop of reaction mixture to a wet pH paper. Reaction was followed by MALDI-MS and HPLC and in most cases it was complete in less than 2 hours. Then DMF was removed by evaporation under vacuum (1 mm Hg) at temperatures less that 30 °C and the residue was dried under vacuum (0.1 mm Hg) overnight.

For deprotection of the cyclic peptide side chains, the dried crude peptide was dissolved in a mixture of TFA/PhOH/H_2O/thioanisole/EDT/TIS (81.5:5:5:2.5:1) (about 100 mL/g of peptide) at room temperature for 1-3 hours. The completion of the reaction was followed by HPLC and MALDI-MS. The TFA solution was concentrated by 5 times by evaporation under vacuum (1 mm Hg), from which the peptide was precipitated by adding it to an ice-cold Et_2O. The purity of dried crude peptides was assessed by HPLC and MALDI-MS. The crude peptides can be partially purified by dissolving in boiling ACN/water/HCl mixture (30/70/0.1) and cooling the turbid solution in a fridge. In case of peptide high solubility in this mixture, the precipitate can be obtained by adding

acetone (3 vol. eq.) to the above solution. Further purification was achieved by preparative reverse phase HPLC (C4, radial compression column, Waters) using, for example, a gradient of eluent A (0.1 % HCl in 99% H_2O/1% ACN (v/v)) and eluent B (0.07 % HCl in 90% ACN/10% H_2O (v/v)) using a flow rate of 24 ml/min.

Combinatorial Peptide Synthesis: Mixture Libraries

Materials for peptide synthesis by Boc chemistry were purchased from a variety of commercial sources. For the synthesis of the first generation libraries of peptides, N-Boc-α-Fmoc-glutamic acid was loaded onto methyl-benzhydrylamine (MBHA) resin through its side chain carboxylate, then the resin was split into four equimolar fractions of 0.25 mmol each for the rest of the synthesis. To each fraction was coupled one of four N-Boc-protected *D*-amino acids, lysine, arginine, glutamic acid, or serine (4 equivalents), using (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-) tetramethyluronimum hexafluorophosphate (HATU, 4 equivalents), 1-hydroxy-7-azabenzotriazole (HOAT, 4 equivalents), and diisopropylethylamine (DIEA, 6 equivalents) in DMF. To positions 3 through 7 were coupled a mixture of N-Boc-protected alanine, leucine, valine, phenylalanine, and tryptophan, at a molar ratio of 1.35: 1.97: 4.47: 1: 1 (10 equivalents versus resin loading) to compensate for the difference in the coupling efficiencies (Pinilla, C.; Appel, J. R.; Blondelle, S. E.; Dooley, C. T.; Eichler, J.; Ostresh, J. M.; Houghten, R. A. "Versatility of positional scanning synthetic combinatorial libraries for the identification of individual compounds." *Drug Dev. Res.* 1994, *33*, 133-145).

Standard procedures were utilized for peptide synthesis except that the odd positions contained amino acids of *L*-chirality and the even positions amino acids with *D*-chirality. In position 8, a mixture of N-Boc-protected *D*-amino acids, lysine, arginine, glutamic acid, and serine, at a molar ratio of 2.24: 2.34: 1.31: 1 was coupled (10 equivalents versus resin loading). After removal of the N-terminal Boc from the peptide chain with neat TFA, the α-Fmoc on the carboxyl terminus of the glutamic acid was removed with 30% piperidine in DMF to allow cyclization of the peptide on the resin, using HATU (2

equivalents), HOAT (2 equivalents), and DIEA (4 equivalents) in DMF/DMSO and/or benzotriazole-1-yl-oxy-trix-pyrrolidino-phosphonium hexafluorophosphate (PyBOP, 1 equivalent), N-hydroxybenzotriazole, (HOBT, 1 equivalent), and DIEA (2 equivalents) in dry 2M LiBr/tetrahydrofuran over a period of 24 - 48 hours. Peptides were cleaved by standard high HF cleavage procedure, washed with ether then extracted with 10% acetic acid followed by DMF. The extracts were pooled and lyophilized.

The specified amino acids of the peptide library sequences showing the greatest biological activity were retained for the generation of the next set of libraries. Subsequent generations of peptide libraries were synthesized in a similar fashion, with the splitting of the resin after the coupling of the specified amino acids determined from the previous generation. The peptides of the combinatorial libraries were identified by electrospray-mass spectrometry (ES-MS) or MALDI-TOF mass spectrometry.

When a peptide library, peptide pool or crude preparation of a peptide showed activity, individual peptides were re-synthesized, HPLC-purified and tested again for activity. For example, a crude preparation of a peptide having a low minimum inhibitory concentration value, *cyclo*[*D*-Arg-*L*-Gln-*D*-Arg-*L*-Trp-*D*-Trp-*L*-Leu-Trp-*L*-Trp] (SEQ ID NO:10), was re-synthesized, HPLC-purified, and tested for anti-microbial activity, and was found to have biological activity similar to the crude.

Single-Compound per Bead Combinatorial Cyclic Peptide Libraries

Cyclic *D, L*-α-peptide combinatorial libraries were prepared using a one-bead-one-compound strategy on macrobeads by a split and pool method. *See,* K.S. Lam, M. Lebl, V. Krchnak, "The 'one-bead-one-compound' combinatorial library method," *Chem. Rev.* 1997, 97, 411-448. Each bead contained a single sequence and was dispersed into microtiter plates using a density of one bead per well. Cleavage of the peptide from a single bead provided about 70-80 μg of peptide per well. This amount of peptide could be used for approximately 100 *in vitro* anti-microbial assays. Mass spectrometric peptide sequencing strategies

were used for rapid identification of selected peptide species within a given library.

Solid-phase peptide synthesis was performed on polystyrene macrobeads functionalized with a TFA-labile trityl linker, which considerably facilitated the synthesis, handling, solid-phase cyclization, and final side chain deprotection and peptide isolation. The growing peptide chain was linked through the first amino acid side chain (for example, lysine or histidine) to the trityl moiety allowing for selective "head-to-tail" cyclization of the completed peptide sequence on solid support. The α-carboxyl group of the first N-α-Fmoc amino acid was protected as an allyl ester. Resin loading and peptide chain elongation was performed under standard Fmoc solid phase peptide synthesis conditions using chlorotrityl polystyrene macrobead resin (500-560 um, Peptides International) as the solid support, with HBTU as a coupling reagent and 20% piperidine in DMF for Fmoc deprotection. After completion of the final amino acid coupling, the resin was exposed to palladium tetrakis(triphenylphosphine) and N-methyl morpholine to remove the C-terminal allyl protecting group. Subsequent N-terminal Fmoc deprotection followed directly by cyclization with PyBop®, provided the desired cyclic peptide generally in high yields. Single macrobeads was dispensed into discrete wells in microtiter plates manually or by using a bead dispenser. The protected cyclic peptide was released from the solid support and deprotected in one step using a 95% TFA (5% cation scavengers) solution. After cleavage, the solvent was removed *in vacuo* to yield cyclic peptide with high purity. Cleavage conditions and work-up procedures were generally optimized to eliminate non-volatile scavengers and potentially deleterious side products from the final peptide. Peptide libraries obtained by the above procedure were sufficiently pure for use in assays.

Materials: Acetonitrile (HPLC grade), dichloromethane (optima grade), dicyclohexylamine (DCHA), diethyl ether (anhydrous), dimethylformamide (sequencing grade), diisopropylethylamine (DIPEA, peptide synthesis grade), and piperidine (anhydrous) were purchased from Fisher and used without further purification. Trifluoroacetic acid (TFA, New Jersey Halocarbon), and 2-(1-*H*-

benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU, Novabiochem), benzotriazole 1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP, Novabiochem) were used without further purification. Tetrakistriphenylphosphine palladium(0) was purchased from Strem Chemicals. Commercially available *N*-Fmoc amino acids for solid-phase peptide synthesis and trityl chloride PS (1% DVB, substitution 0.5 – 1.05 mmol g^{-1}) resin were used as obtained from Novabiochem or Bachem. Trityl chloride macrobead resin was obtained from Peptides International.

Preparation of Fmoc-Lysine(Boc)-Oallyl. Fmoc-Lysine(Boc)-OAllyl was made according to the protocol of Kates, S. A.; Solé, N. A.; Johnson, C. R.; Hudson, D.; Barany, G.; Albericio, F. *Tetrahedron Lett.* 1993, *34*, 1549–1552. Fmoc-Lys-(Boc)-OH (5 g, 10.6 mmol) was added to allyl bromide (25 mL, 0.29 mol), followed by DIPEA (3.73 mL). This mixture was heated at 90 °C for 1 h. The reaction was allowed to cool, concentrated by rotary evaporation, and after dilution with ethyl acetate was washed with 2 × 0.1 N HCl, 2 × saturated sodium bicarbonate at pH < 9.5, followed by brine. The organic layer was filtered through a pad of silica gel and concentrated to afford a solid. This solid was washed with ether to provide a white powder that was used directly in the next step.

Deprotection of Side Chain Boc Protecting Group. An appropriate amount of Fmoc-Lys(Boc)-OAllyl for a resin loading of 0.5 mmol g^{-1} was placed in a round bottom flask. Sufficient dichloromethane to dissolve the solid was added followed by an equivalent amount of TFA. After stirring for 1 h the solution was evaporated and the residue of Fmoc-Lys-OAllyl was dried *in vacuo*.

Resin loading. Trityl chloride resin was swollen in dry deacidified (Na_2CO_3) dichloromethane for 20 min. A solution of Fmoc-Lys-OAllyl in dichloromethane was added to the resin, immediately followed by 4 eq. of DIPEA. After shaking for 2 hours the resin was washed resin with dichloromethane, then shaken with 10% MeOH: 10% DIPEA: 80% dichloromethane for 10 min. After washing with dichloromethane and drying *in*

vacuo the resin loading was evaluated based on Fmoc released monitored by UV absorption at 290 nm.

Peptide Synthesis. Peptides were synthesized using standard solid-phase Fmoc protocols (see Wellings, D. A.; Atherton, E. *Methods Enzymol.* 1997, *289*, 44–67) on the Fmoc-Lys-OAllyl loaded trityl resin. Following synthesis of the linear peptide the resin was swollen in dry dichloromethane for 20 min. To the resin was added a degassed solution of 0.5 eq. $Pd(PPh_3)_4$ in 90% $CHCl_3$: 10% 4-methylmorpholine. After shaking under Argon for 5 hours the resin was washed with a solution of 1% sodium dimethylthiocarbamic acid in DMF (3 × 2 min), 1% DIPEA (3 × 2 min) in DMF. After the final Fmoc deprotection (25% piperidine in DMF, 2 × 10 min), the resin was washed thoroughly with DMF (3 × 3 min), 10% DIPEA/DMF (3 × 3 min), 0.8 M LiCl/DMF (3 × 3 min). The resin was treated with 5 eq. PyBOP, 5 eq. HOAt, 20 eq. DIPEA in 0.8 M LiCl/DMF for at least 12 hours. After washing with DMF (3 × 3 min), DCM (2 × 3 min) followed by MeOH the peptide was cleaved from the resin and deprotected with 2.5% TIS: 2.5% H_20: 95% TFA. Peptides were recovered by precipitation with ether or by evaporation of the cleavage mixture.

EXAMPLE 2

Anti-Cancer Cyclic Peptide Agents

Preparation of peptide stock solutions

1-1.5 mg of peptide was weighed into a 1.5 ml Eppendorf tube. To this tube were added 50 µl of DMSO and 950 µl of a 9% sucrose/water solution. The solution was vortexed and sonicated (if necessary) to completely dissolve peptide. Concentrations of stock solutions of peptides containing tryptophan residues were quantitatively determined by UV absorbance at 280 nm and adjusted to 1 mg/ml. Concentrations of stock solutions of peptides without tryptophan were approximated from the mass of peptide added.

Anti-Cancer Cytotoxicity Determination – trypan blue:

180 µl of cells at the appropriate concentration were placed in each well of a 96-well plate. After 24 hr, 20 µl of peptide stock solution was added to each

well. After the determined exposure time to peptide, media was removed and 90 µl of HBS buffer was added. 10 µl of trypan blue was then added to each well. After 10 min, the cells were counted under a light microscope and the percent viable cells in each well was recorded. LC_{50} values were determined from this percentage.

Anti-Cancer Cytotoxicity Determination – MTT:

180 µl of cells at the appropriate concentration were placed in each well of a 96-well plate. After 24 hr, 20 µl of peptide stock solution was added to each well. After the determined exposure time to peptide, media was removed and 90 µl of fresh media was added. 10 µl of 0.2% 3-[4,5-dimethylthiazol-α-yl]- α, 5-diphenyltetrazolium bromide (MTT) in saline was added to each well. Cells were incubated for 2 hours. Media was then removed, and 100 µl of DMSO was added to each well. Optical density at 560 nm was measured in an ELISA plate reader. The well showing OD_{560} equal to half the value for cells that received no peptide was taken as the LC_{50}.

Assay Conditions

Before screening larger libraries of peptides, five test peptides were chosen to use in a preliminary study. The goal of this study was to work out the details of the assay conditions prior to screening large libraries. Cytotoxicity studies were first performed using a K44A HeLa cell line, a HeLa cell line that overexpressed a K44A mutant dynamin under the control of tetracycline-regulated promoter. Cells grown in the presence of tetracycline (Tet+) expressed wild type dynamin, while cells grown without tetracycline (Tet-) overexpressed the K44A mutant dynamin. Because the peptides were being tested for anti-viral activity at the same time, some cells were also exposed to Adenovirus.

Initial data were based on a trypan blue exclusion assay. In this assay, a dye was introduced to a well containing the test cells. Healthy cells do not uptake the dye and remain colorless, while dead or dying cells turn a dark blue color. Table 3 below summarizes results from Tet+ HeLa cells exposed to peptide for 48 hours.

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TABLE 3

SEQ ID NO:	sequence	LC_{50} (μg/ml)
54	c[KSKLFLFL]	25
50	c[KHKLWLWL]	13
61	c[KSKLWLWL]	6
133	c[KRWLWL]	6
12	c[KSKWLWLW]	1.5

Thus, a peptide having SEQ ID NO:12 showed toxicity against these HeLa cells at concentrations above 1.5 μg/ml. This peptide is currently in animal testing phase for antimicrobial activity and shows very low *in vivo* toxicity.

In an effort to more closely approximate conditions to be used in the assay, the experiment was repeated with peptide having SEQ ID NO:12, this time with a 4 hour exposure with and without tetracycline and Adenovirus (Ad). The results are summarized below in Table 4.

TABLE 4

	Peptide SEQ ID NO:12 (4 hr exposure)			
Tet	+	+	-	-
Ad	+	-	+	-
LC_{50} (μg/ml)	1.5	1.5	1.5	1.5

The toxicity against HeLa cells remained unchanged in the four conditions, whether or not tetracycline or Adenovirus was present. Additional cytotoxicity assays were carried out on Tet+ HeLa cells as follows.

In order to make sure that the trypan blue exclusion assay accurately reflected anti-cancer cell toxicity, and not simply that the peptide allowed the dye into the cell without killing it, the toxicity determination was repeated for peptide SEQ ID NO:12 using 3-[4,5-dimethylthiazol-α-yl]- α, 5-diphenyltetrazolium bromide (MTT) to detect whether cells exhibited metabolic activity. This assay relies on metabolic activity of cells to reduce MTT into a

detectable dye. MTT was added to the cell media, and the cells were incubated for four hours. They were then washed and the insoluble dye resulting from any MTT reduced by the cells was dissolved in DMSO. The optical density of the cells was then measured (560 nm). This optical density was proportional to the population of living cells in a sample. The levels of toxicity against the HeLa cell line using the MTT assay was similar to the trypan blue exclusion assay (1.5-3 μg/ml).

Different exposure times to the SEQ ID NO:12 peptide were then tested. These results are summarized in Table 5.

TABLE 5

Exposure time (hr)	LC_{50} (μg/ml)
0.5	6
1	6
2	3
4	1.5
6	1.5

Three other cell lines were tested using the peptide having SEQ ID NO:12 and four hour exposure times: wild type HeLa cells (human cervical cancer cells), A549 cells (human lung epithelial carcinoma cells) and Chang C cells (human conjunctival epithelial cells). In each case, the LC50 was 6 μg/ml. The results are summarized below in Table 6.

TABLE 6

Cell line	LC_{50} (μg/ml)
HeLa WT	6
A549	6
Chang C	6

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Larger Cancer Cell Screen

Peptide SEQ ID NO:12 was then screened against a panel of 60 human tumor cell lines. Cells were exposed to peptide for a 48 hour period and cell viability was estimated using a sulforhodamine B assay. For additional details concerning this screen see the following Examples and the website at http://dtp.nci.hih.gov. The peptide was tested at five 10-fold dilutions and percent growth was calculated relative to control cells that received no peptide (growth of control cells for 48 hours was taken as 100% growth). Results of this assay indicated that peptide SEQ ID NO:12 showed strong activity against two leukemia cell lines (HL-60 and MOLT-4). The data are summarized in Figures 9-15 and Table 7 below.

TABLE 7

Cell line	GI_{50} (μg/ml)
HL-60 (TB)	0.019
MOLT-4	0.010

This *in vitro* anti-tumor screen consisted of 60 human tumor cell lines against which peptide SEQ ID NO:12 was tested at a minimum of five concentrations using 10-fold dilutions. A 48 hour continuous drug exposure protocol was used, and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The data are summarized in Figures 9-15.

EXAMPLE 3

Anti-Cancer Cyclic Peptide Agents

Cyclic peptides were evaluated for anti-cancer activity against breast cancer, lung cancer, and CNS tumor cell lines maintained by The Developmental Therapeutics Program of the National Cancer Institute (NCI). Cyclic peptides were tested at 20 ug/mL against three cell lines as described in the website at dtp.nci.nih.gov/branches/btb/ivclsp.html. Cell lines tested were MCF7 (tumor type: breast), NCI-H460 (tumor type: non-small cell lung), and SF-268 (tumor type: CNS).

Results for single-point cytotoxicity testing. Four 96-well plates were used for testing. Each cell line was inoculated and pre-incubated on a microtiter plate. Test agents were then added at a single concentration and the culture incubated for 48 hours. End-point determinations were made with alamar blue (*Biotechniques* 21(5) 780-782 (1996)). Results for each test agent were reported as the percent of growth of the treated cells when compared to the untreated control cells. Percent growth of 32 or less in any one cell line was considered a "pass" for the test well. Each cyclic peptide submitted was tested at 20 ug/mL. The cyclic peptides tested are set forth in Table 8, including 22 synthesized cyclic D,L-alpha peptides, 11 peptides having from four to six beta amino acids, 160 peptides from library Hexa1 (crude), and 160 peptides from library Octa1 (crude).

The Hexa1 cyclic peptides included peptides having the sequence cyclic[KYZZYX] wherein X represents amino acids Lys, His, Arg, Ser, Asn, Glu; Y represents amino acids Lys, His, Arg, Ser, Asn, Glu, Trp, Leu; Z represents amino acids Trp, Leu; K is Lys; and underlined amino acids are D-amino acids while non-underlined amino acids are L-amino acids. The Octa1 cyclic peptides included peptides having the sequence cyclic[KXYZZZYX] wherein X represents amino acids Lys, His, Arg, Ser, Asn, Glu; Y represents amino acids Lys, His, Arg, Ser, Asn, Glu, Trp, Leu; Z represents amino acids Trp, Leu; and K is Lys. Crude Hexa1 and Octa1 library materials were used for testing. Cyclic peptides from the Hexa1 library having the least target activity in this assay ([KHKWWW] and [NKHLLL] or [HKNLLL]) were not re-synthesized or purified for additional activity testing.

TABLE 8. Cyclic peptides submitted and hit rate.

Peptide category	Number submitted	Number of hits	Hit rate (%)
ATI 0001001-0001022	22	9	41%
Non-D,L-alpha peptides	11	2	18%
Crude Hexa1 library	160	2	1%
Crude Octa1 library	160	0	0%

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Table 9 shows the sequences of submitted cyclic peptides having cancer cell cytotoxicity in the above-described assay for at least one of three cell lines at 20 ug/mL. A shorthand for peptide sequences was employed: the one-letter amino acid symbol was used but underlining indicates that the amino acid is a D-amino acid; non-underlining indicates the amino acid is a L-amino acid. Where β-amino acids are present a lower case "h" is used before the one letter amino acid symbol for the related α-amino acid.

The MCF-7 cell line was most sensitive to the tested peptides (12 peptides having target activity in this assay). The NCI-H460 and SF-268 cell lines were less sensitive (4 and 5 peptides having target activity in this assay against these lines, respectively). Of the 13 cyclic peptides, in only one case was there target activity with the NCI-H460 cell line without corresponding target activity in the MCF-7 cell line. In no cases was there target activity against SF-268 without corresponding activity in the MCF-7 cell line. One cyclic peptide was included in the list of hits that showed a growth of 35% for MCF7, just over the target 32% toxicity for this experiment. Of the cyclic non-D,L-alpha peptides tested, two were active below 32% growth for at least one cell line in this experiment.

In summary, the peptides tested were differentially active against the MCF7 (tumor type: breast), NCI-H460 (tumor type: non-small cell lung), and SF-268 (tumor type: CNS) cell lines. Hence, the peptides were not indiscriminately cytotoxic, but exhibited some selectivity in their action against different cancer cells.

TABLE 9. Peptide sequences and cancer cell cytotoxicity activity (% growth).

Sequence*	MCF7	NCI-H460	SF-268
[KHKWWW]	9	79	70
c-[hK-hK-hW-hL-hW-hL-]	0	0	0
[NKHLLL]	86	7	44
[HSHKWLWK] (88)	30	62	35

[SKSKWLWK] (91)	2	41	23
[SKKWLWLK] (77)	19	99	75
[SSSKWLWK] (93)	0	94	16
[SKKLWLWK] (145)	5	80	55
[HKHKWLWK] (85)	19	83	50
[QKKLWLWK] (146)	35	88	86
[QKRLWLWK] (147)	8	76	46
c-[hR-hK-hK-hL-hL-hW-]	1	22	32
[HRWLWRHK] (148)	1	3	33

* h represents the beta analog of the parent amino acid

Analysis of 22 cyclic D,L-alpha peptides. Test results from the 22 synthesized cyclic D,L-alpha peptides showed that all but four had cancer cell cytotoxic activity in these assays (Table 10). Moreover, the amount of cyclic peptide used to achieve cancer cell cytotoxicity (20 ug/ml) was substantially less than the amount that caused hemolysis.

Table 10. Cytotoxicity of Cyclic D,L-alpha peptides

Sequence (SEQ ID NO:)	MCF7	NCI-H460	SF-268	Hemolysis HD50 (ug/ml)
KSKKFLFL (76)	73	80	75	146
KSKKWLWL (77)	19	99	75	>200 (13%)
KSKKLWLW (149)	5	80	55	>200 (12%)
KHKLWLWL (50)	97	103	102	147
KHKRWLWL (150)	63	100	92	140
KHKHKWLW (85)	19	83	50	>200 (21%)
KHQHKWLW (87)	97	87	76	>200 (35%)
KHSHKWLW (88)	30	62	35	145
KSKSKWLW (91)	2	41	23	195
KSSSKWLW (93)	0	94	16	155
KHKHFLWL (72)	129	98	101	>200 (25%)
KHKLFLAL (47)	132	99	98	>200 (2%)
RHKHRWLW (151)	107	105	106	>200 (0%)
KKKWLWLW (17)	67	76	84	180
KQKKLWLW (152)	35	88	86	>200 (12%)

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Sequence (SEQ ID NO:)	MCF7	NCI-H460	SF-268	Hemolysis HD50 (ug/ml)
KQRWLWLW (8)	82	91	90	160
RSKLWLWL (153)	84	95	100	150
KSKWLWLW (12)	58	73	70	>200 (0%)
RRKWLWLW (18)	57	94	89	
KHKHLWLW (154)	66	107	78	160
KQKRLWLW (155)	8	76	46	160
RHKHRWLW (156)	1	3	33	>200 (8%)

Sequence analysis indicated that there were more hydrophilic amino acid residues than hydrophobic amino acid residues in the peptides designated "hits" in this assay (41 versus 31) and that there were less hydrophilic amino acid residues than hydrophobic amino acid residues in the cyclic peptides designated "non-hits" in this assay (47 versus 57). These data are summarized in Table 11.

TABLE 11. Counts of hydrophilic and hydrophobic residues.

Residue type	Number in peptides with target cytotoxicity	Number in peptides without target cytotoxicity	Total
Hydrophilic	41	47	88
Hydrophobic	31	57	88
Total	72	104	

The counts of individual hydrophilic residues were normalized relative to the total count of all the hydrophilic residues in the cyclic peptides that showed target activity in this assay and the total submitted cyclic peptides, respectively. Differences in percent representation in the cyclic peptides having target activity in this assay and the total cyclic peptides submitted highlight differences in cytotoxicity selection. Serine residues were represented more heavily in the cyclic peptides having cancer cell target activity in this assay (20%) than in the total tested cyclic peptides (13%). Lysine residues were slightly more

represented in the cyclic peptides having target activity in this assay than in the initial distribution (54% v 52%). Conversely, His and Arg were less represented in the cyclic peptides having target activity in this assay (His: 15% v 19%, Arg: 7% v 11%) (Table 12).

TABLE 12. Counts of individual residues compared to overall counts.

Residue	Count in peptides with target cytotoxicity	Percent (relative to 41)	Overall Count	Percent (relative to 88)
K	22	54	46	52
H	6	15	17	19
S	8	20	11	13
R	3	7	10	11
Q	2	5	4	5

In Table 13, the hydrophilic-hydrophobic motif and activity were compared. A greater number of hydrophilic residues appeared to correlate with greater cytotoxic activity in this experiment. Specifically, 71% of the five hydrophilic-three hydrophobic (5-3) cyclic peptides were active at the target level, whereas only 50% of the 4-4 cyclic peptides were active at the target level, and 0% of the 3-5 cyclic peptides were active at the target level. Of the cyclic peptides active at the target level, 56% had a 5-3 motif, 44% had a 4-4 motif, and 0% had a 3-5 motif.

TABLE 13. Analysis of hydrophilic-hydrophobic motif and activity

Motif hydrophilic-hydrophobic	total number	# in actives	% with motif that were active	% of active peptides that had each motif
3-5	7	0	0	0
4-4	8	4	50	44
5-3	7	5	71	56

As indicated in Table 14, all cyclic peptides tested were also analyzed by motif as well as the presence of serine residues. Within each motif, the number

of cyclic peptides with 1,2,3, or zero serine residues were tabulated. The asterisk denotes a cyclic peptide that showed activity at the target level in this assay. A greater number of hydrophilic residues and more serine residues correlated with increased cytotoxic activity.

TABLE 14. Analysis of motif and presence of Serine

Motif hydrophilic-hydrophobic	Total # with 1 Ser	Total # with 2 Ser	Total # with 3 Ser	Total # with 0 Ser
3-5	2	0	0	5
4-4	3**	0	0	5**
5-3	1*	1*	1*	4**
*=active, **= two active				

Table 15 provides the percentage growth of breast, non-small cell lung and CNS cancer lines in the presence of 20 ug/mL cyclic D,L-beta peptides. The "h" indicates that the amino acid is a beta amino acid. As shown in Table 15, beta-peptides c-[hK-hK-hW-hL-hW-hL-] and c-[hR-hK-hK-hL-hL-hW-] (sequences cyclic [β-Lys- β-Lys- β-Trp- β-Leu- β-Trp- β-Leu] or cyclic [β-Arg- β-Lys- β-Lys- β-Leu- β-Leu -β-Trp], respectively) are highly effective at suppressing cancer cell growth.

TABLE 15. Beta-amino acid peptide test results

Sequence	(Breast)	(Non-Small Cell Lung)	(CNS)
	MCF7	NCI-H460	SF-268
c-[hK-hK-hL-hW-]	103	109	102
c-[hK-hK-hW-hL-hW-]	91	55	93
c-[hK-hK-hW-hL-hW-hL-]	0	0	0
c-[hK-hK-hW-hL-hW-]	113	87	90
c-[hK-hS-hK-hF-hL-hF-]	121	88	99
c-[hK-hN-hK-hW-hL-hW-]	104	104	117
c-[hK-hR-hN-hL-hW-]	107	116	101
c-[hK-hR-hN-hW-hL-hW-]	111	110	106
c-[hK-hR-hN-hR-hL-hW-]	106	112	104
c-[hR-hK-hK-hL-hW-]	105	107	103
c-[hR-hK-hK-hL-hL-hW-]	1	22	32

EXAMPLE 4

Anti-Cancer Cyclic Peptide Agents

Twelve peptides were evaluated for anti-cancer activity against 60 cell lines maintained by The Developmental Therapeutics Program of the National Cancer Institute (NCI). The peptides included the following eleven cyclic peptides and one linear peptide: [KSKKLWLW] (SEQ ID NO:149), [KSSSKWLW] (SEQ ID NO:91), [KHKHFLWL] (SEQ ID NO:72), [KHKLFLAL] (SEQ ID NO:47), [RHKHRWLW] (SEQ ID NO:151), [KWKWSWLW] (SEQ ID NO:107), [SEKHKLWW] (SEQ ID NO:157), [KKKRHLWL] (SEQ ID NO:158), -KSKWLWLW-(linear) (SEQ ID NO:159), [KRKWLW] (SEQ ID NO:125), [KSKWLW] (SEQ ID NO:126), and [KSKWLWLW] (SEQ ID NO:12).

Procedures

Human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter

plates in 100 µL at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental peptides were solubilized in dimethyl sulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of peptide addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, ten-fold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following peptide addition, the plates were incubated for an additional 48 h at 37°C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates are incubated for 10 minutes at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain is subsequently solubilized with 10 mM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm.

For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth

in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

Three dose response parameters were calculated for each experimental agent. Growth inhibition of 50 % (GI50) was calculated from [(Ti-Tz)/(C-Tz)] x 100 = 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from Ti = Tz. The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from [(Ti-Tz)/Tz] x 100 = -50. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested. Further procedures can be found at the website at dtp.nci.nih.gov/branches/btb/ivclsp.html.

Results

As indicated in chart provided in Figure 16, six of the eleven cyclic peptides had GI_{50} values in the micromolar range. As expected, the linear peptide, -KSKWLWLW- was not active at such low concentrations.

EXAMPLE 5

Procedures for Assessing Activity as Anti-Microbials

Cell membrane selectivity has also been assessed for various peptides of the invention to ascertain whether the peptides would show antimicrobial activity against prokaryotic cellular membranes as opposed to eukaryotic cellular membranes.

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Antimicrobial activity can be determined using a broth dilution assay essentially as descried in the guidelines of the National Committee for the Control of Laboratory Standards (NCCLS) [National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.* Fourth edition. Approved Standard (1997). Document M7-A4. (NCCLS, Villanova, Pennsylvania, 1997]. Test tubes (macrodilution method) or microtiter plates (microdilution method) containing two-fold serial dilution of peptides were inoculated with various bacterial cultures. Controls included non-inoculated medium (sterility), vehicle control, and various commercially available antibiotics for which minimal inhibitory concentrations against tested organisms were known. Several of the strains of bacteria tested are described in Table 16.

Table 16

Species	Strain	Type	Special Properties
Bacillus cereus	ATCC 11778	Gram positive	
Enterococcus faecalis	ATCC 51299	Gram positive	Vancomycin resistant (vanB); resistant to vancomycin, gentamicin and streptomycin.
Enterococcus faecium	SP180	Gram positive	Vancomycin resistant (vanA); resistant to multiple antibiotics
Staphylococcus aureus	OS2	Gram positive	
Staphylococcus. aureus	ATCC 33591	Gram positive	Methicillin-resistant.
Staphylococcus. aureus	ATCC 33592	Gram positive	Methicillin-resistant; resistant to multiple antibiotics
Streptococcus pneumoniae	ATCC 6301	Gram positive	
Listeria monocytogenes	ATCC 19115	Gram positive	
Escherichia coli	K12	Gram negative	Reference strain
Escherichia coli	JM109 (DE3)	Gram negative	
Escherichia coli	EL744 K12 (tolC::kan)	Gram negative	Lacks key efflux pump component
Hemophilus. influenzae	ATCC 49247	Gram negative	Reference strain
Salmonella typhimurium	ATCC 14028	Gram negative	

Stock peptide solutions were prepared in 5% DMSO in aq. sucrose (9%). Determination of peptide concentrations was done by quantitative HPLC analysis using known concentrations of internal standards and/or by measuring UV absorption of tryptophan--containing peptide solutions in H_2O (λ=280 ε_{Trp}: 5690). Serial 2-fold dilutions were made in the above DMSO/aq. sucrose (9%) mixture with concentrations ranging approximately 400-2 μg/ml and aliquots were dispensed in test tubes (100 μl) for the macrodilution test or in microtiter plates (20 μl) for the microdilution assays. Overnight cultures of different microorganisms grown in suitable media were diluted 4000 times to an approximate inoculum size of 2.5×10^5 cfu/ml. Macrodilution methods were performed using procedures similar to those described in the National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.* Fourth edition. Approved Standard (1997). Document M7-A4. (NCCLS, Villanova, Pennsylvania, 1997). Two ml aliquots of the above inoculum were dispensed to test tubes containing different peptide solutions. After incubation at 37 °C with shaking for 18 hours the lowest concentration at which no bacterial growth was observed was recorded as the MIC. Microdilution methods were performed using procedures similar to those described in the National Committee for Clinical Laboratory Standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.* Fourth edition. Approved Standard (1997). Document M7-A4. (NCCLS, Villanova, Pennsylvania, 1997. Eighty μl aliquots of the above inoculum were dispensed into 96-well microtiter plates containing different peptide solutions and incubated for 18 h at 37 °C with shaking (plates were sealed with parafilm to avoid excessive evaporation of culture medium) and MIC's were recorded. Each assay was performed at least twice and errors were typically ± one dilution.

Minimum bactericidal concentrations (MBC) were determined according to the guidelines of the National Committee for Clinical Laboratory Standards

(NCCLS). National Committee for Clinical Laboratory Standards. Methods for Determining Bactericidal Activity of Antimicrobial Agents. Approved Standard (1999). Document M26-A. (NCCLS, Villanova, Pennsylvania, 1999). Fifty µl aliquots from MIC, 2xMIC and 4xMIC assay wells were removed and plated in antibiotic-free agar plates by using the lawning technique. Growth and sterility controls were sampled in the same manner. The lawned plates were incubated for 24-48 hours and the MBC was determined as the lowest concentration at which 99.9% killing was achieved.

Membrane depolarization was monitored by a change in fluorescence emission intensity of the cyanine membrane potential-sensitive dye 3,3'-dipropylthiadicarbocyanide iodide ($diSC_3$). Intact *S. aureus* were grown at 37 °C with agitation to mid log-phase ($O.D._{600} = 0.5$). Cells were centrifuged and washed once with buffer (20 mM glucose, 5 mM HEPES pH 7.3) and resuspended to an $O.D._{600}$ of 0.05 in a similar buffer containing 0.1 M KCl. Cells were incubated with 1 µM $diSC_3$ until a stable reduction of fluorescence was achieved (approximately 15 min), indicating incorporation of the dye into the bacterial membrane. Peptides were added from stock solutions (1 mg/ml) dissolved in 5% DMSO in aqueous sucrose (9%) to achieve desired 0.1 to 10xMIC concentrations.

Selectivity of cyclic peptides for bacterial over normal mammalian cells was evaluated by measuring red blood cell hemolytic activities as described in Tosteson, M.T. Holmes, S.J., Razin, M. and Tosteson, D.C. Melittin Lysis of Red Cells, J. *Membrane Biol. 87,* 35-44(1985). Heparinized murine blood was centrifuged at 1000 x g for 10 minutes. The supernatant and the buffy coat were removed. Erythrocytes were washed three times with 0.9% saline solution and then resuspended to a concentration of 5 % in saline containing 10% FBS (v/v). Red blood cells were then treated with serial dilutions of test peptides in a 96 well plate at 37 °C for 30 minutes. Control samples included a saline solution and 1 % Triton X-100 as 0 and 100 % hemolysis, respectively. In some cases, mellitin (a linear peptide that is hemolytic against mammalian red blood cells *in vitro* at a concentration of about 10 µg/ml) was used as a further control. Plates

were centrifuged at 1000 g for 10 minutes. Aliquots of the supernatant were diluted 2 times with saline solution and the absorbance was measured at 560 nm.

Stock peptide solutions for in vivo experiments were prepared in aqueous sucrose (9%). To facilitate dissolution of the peptide, the initial suspension was sonicated for 15-20 min. The obtained solution was sterilized by passage through a sterile 0.45μm filter (COSTAR, μStar, Corning Inc.). Determination of peptide concentrations was done by UV absorption in H_2O (λ=280 ε_{Trp}: 5690 $cm^{-1}M^{-1}$ for tryptophan-containing peptides) of different aliquots and found to be 60-70% of the one corresponding to w/v. Alternatively, the concentrations of various peptide stock solutions were determined by quantitative HPLC analysis using known concentrations of internal standards. Further the peptide solutions were appropriately diluted with sterile aq. sucrose (9%).

Bacteria were prepared for *in vivo* testing using procedures similar to those as previously described. *See* V. Lorian, Antibiotics in Laboratory Medicine, Williams and Wilkins, Baltimore 1991. *S. aureus* MRSA bacteria (ATCC 33591) were grown at 37 °C in 5 mL of Antibiotic Medium-3 (AM-3, Difco Laboratories) with agitation for 12 hours to a stationary phase. Cells were collected by centrifugation, washed twice with saline solution and resuspended in about 10 mL of saline to an $O.D._{650}$ of 1.2. This suspension was diluted ten times in a sterilized 5% mucin (Difco) in saline to a concentration of 2-$4x10^7$cfu/ml (actual inoculum size was verified by colony counts on agar plates).

Vancomycin resistant *Enterococcus faecium* (VREF) bacteria (ATCC 51575) were grown at 37 °C in 60 mL of Brain Heart Infusion medium (BHI, Difco Laboratories) with agitation for 16 hours to a stationary phase. Cells were collected by centrifugation, washed twice with saline solution and resuspended in 6 mL of saline to a theoretical $O.D._{650}$ of 9-10 (determined by taking $OD._{650}$ of 10-20 fold diluted samples and correlating it to the original concentration). This suspension was diluted 15 times in a sterilized 5% mucin (Difco) in saline to a concentration of 5-$7x10^8$cfu/ml (actual inoculum size was verified by colony counts on agar plates).

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EXAMPLE 6

In Vitro Selectivity for Different Cell Types

The selectivity of different cyclic peptides for different cell membranes is illustrated in this Example by *in vitro* antibacterial assays against a variety of bacteria. The first cyclic peptide tested had sequence Lys-D-Gln-Arg-D-Trp-Leu-D-Trp-Leu-D-Trp (SEQ ID NO:9). This cyclic peptide (SEQ ID NO:9) displayed potent activity against gram positive *Bacillus subtilis* and *Staphylococcus aureus* as well as against gram negative *Streptococcus pneumonieae* and vancomycin-resistant *Enterococcus faecalis* (Table 17).

Table 17. *In vitro* activity of Lys-D-Gln-Arg-D-Trp-Leu-D-Trp-Leu-D-Trp (SEQ ID NO:9)

Gram positive bacteria	MIC[a] (μg/ml)
Bacillus subtilis[c]	3[b]
Bacillus cereus[d]	2
Staphylococcus aureus[e]	3[b]
Listeria monocytogenes[i]	20
Enterococcus faecalis[g]	10[b]
Streptococcus pneumoniea[h]	10[b]
Gram negative bacteria	**MIC (μg/ml)**
Salmonella typhimurium[i]	>70
Mammalian cells	
Red blood cell (mouse)	45[j]
M21 (melanoma)	>75[k]

[a] Minimum inhibitory concentration; [b] Data from MDS Panlabs Pharmacology Services; [c] ATCC 43223; [d] ATCC 11778; [e] ATCC25923; [f] ATCC19115; [g] vancomycin-resistant clinical isolate; [h] erythromycin- and ampicillin-resistant clinical isolate; [i] ATCC 14028; [j] Hemolytic dose where 50% of red blood cells are hemolyzed (HD_{50} (μg/ml)); [k] LD_{50} (μg/ml).

The six- and eight-residue amphiphilic cyclic peptides set forth in Table 18 were made to further probe the activity of these peptides and to examine the

relationship between activity, surface properties, and membrane selectivity. Peptides were synthesized by standard solid-phase BOC or FMOC synthetic protocols, cyclized in solution or on solid support, purified by RP-HPLC, and characterized by MALDI-TOF or ESI-mass spectrometry. Underlining indicates that amino acid is a D-amino acid residue.

Table 18: Peptide Sequences

SEQ ID NO:	Shorthand	Sequence Listing	Xaa Definition
1 – cyclic	QAQAQAQAQAQA	Gln-Xaa$_1$-Gln-Xaa$_1$-Gln-Xaa$_1$-Gln-Xaa$_1$-Gln-Xaa$_1$-Gln-Xaa$_1$	Xaa$_1$ = D-Ala
2 – cyclic	QLWLWLWL	Gln-Xaa$_1$-Trp-Xaa$_1$-Trp-Xaa$_1$-Trp-Xaa$_1$	Xaa$_1$ = D-Leu
3 – cyclic	KLWLWLWL	Lys-Xaa$_1$-Trp-Xaa$_1$-Trp-Xaa$_1$-Trp-Xaa$_1$	Xaa$_1$ = D-Leu
4 – cyclic	KLKLKLKL	Lys-Xaa$_1$-Lys-Xaa$_1$-Lys-Xaa$_1$-Lys-Xaa$_1$	Xaa$_1$ = D-Leu
5 – cyclic	SKSWLWLW	Xaa$_1$-Lys-Xaa$_1$-Trp-Xaa$_2$-Trp-Xaa$_2$-Trp	Xaa$_1$ = D-Ser Xaa$_2$ = D-Leu
6 – cyclic	THSWLWLW	Xaa$_1$-His-Xaa$_2$-Trp-Xaa$_3$-Trp-Xaa$_3$-Trp	Xaa$_1$ = D-Thr Xaa$_2$ = D-Ser Xaa$_3$ = D-Leu
7 – cyclic	RGDWLWLW	Xaa$_1$-Gly-Xaa$_2$-Trp-Xaa$_3$-Trp-Xaa$_3$-Trp	Xaa$_1$ = D-Arg Xaa$_2$ = D-Asp Xaa$_3$ = D-Leu
8 – cyclic	KQRWLWLW	Xaa$_1$-Gln-Xaa$_2$-Trp-Xaa$_3$-Trp-Xaa$_3$-Trp	Xaa$_1$ = D-Lys Xaa$_2$ = D-Arg Xaa$_3$ = D-Leu
9 – cyclic	KQRWLWLW	Lys-Xaa$_1$-Arg-Xaa$_2$-Leu-Xaa$_2$-Leu-Xaa$_3$	Xaa$_1$ = D-Gln Xaa$_2$ = D-Trp
10 – cyclic	RQRWLWLW	Xaa$_1$-Gln-Xaa$_1$-Trp-Xaa$_2$-Trp-Xaa$_2$-Trp	Xaa$_1$ = D-Arg Xaa$_2$ = D-Leu
11 – cyclic	KQKWLWLW	Xaa$_1$-Gln-Xaa$_1$-Trp-Xaa$_2$-Trp-Xaa$_2$-Trp	Xaa$_1$ = D-Lys Xaa$_2$ = D-Leu
12 – cyclic	KSKWLWLW	Xaa$_1$-Ser-Xaa$_1$-Trp-Xaa$_2$-Trp-Xaa$_2$-Trp	Xaa$_1$ = D-Lys Xaa$_2$ = D-Leu
13 – cyclic	SHKWLWLW	Xaa$_1$-His-Xaa$_2$-Trp-Xaa$_3$-Trp-Xaa$_3$-Trp	Xaa$_1$ = D-Ser Xaa$_2$ = D-Lys Xaa$_3$ = D-Leu
14 – cyclic	SKHWLWLW	Xaa$_1$-Lys-Xaa$_2$-Trp-Xaa$_3$-Trp-Xaa$_3$-Trp	Xaa$_1$ = D-Ser Xaa$_2$ = D-His Xaa$_3$ = D-Leu

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SEQ ID NO:	Shorthand	Sequence Listing	Xaa Definition
15 – cyclic	SHHWLWLW	Xaa_1-His-Xaa_2-Trp-Xaa_3-Trp-Xaa_3-Trp	Xaa_1 = D-Ser Xaa_2 = D-His Xaa_3 = D-Leu
16 – cyclic	EKHWLWLW	Xaa_1-Lys-Xaa_2-Trp-Xaa_3-Trp-Xaa_3-Trp	Xaa_1 = D-Glu Xaa_2 = D-His Xaa_3 = D-Leu
17 – cyclic	KKKWLWLW	Xaa_1-Lys-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
18 – cyclic	RRKWLWLW	Xaa_1-Arg-Xaa_2-Trp-Xaa_3-Trp-Xaa_3-Trp	Xaa_1 = D-Arg Xaa_2 = D-Lys Xaa_3 = D-Leu
19 – cyclic	KRKWLWLW	Xaa_1-Arg-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
20 – cyclic	RRRWLWLW	Xaa_1-Arg-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Arg Xaa_2 = D-Leu
21 – cyclic	HKHWLWLW	Xaa_1-Lys-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-His Xaa_2 = D-Leu
22 – cyclic	KHKWLWLW	Xaa_1-His-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
23 – linear	WKKKWLWLW	Trp-Xaa_1-Lys-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
24 – linear	KKKWLWLW	Xaa_1-Lys-Xaa_1-Trp-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
25 – linear	Ac-KKKWLWLW-$CONH_2$	Xaa_1-Lys-Xaa_2-Trp-Xaa_3-Trp-Xaa_3-Xaa_4	Xaa_1=Ac-D-Lys Xaa_2 = D-Lys Xaa_3 = D-Leu Xaa_4 = D-Trp-$CONH_2$
26 – cyclic	KKLWLW	Xaa_1-Lys-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
27 – cyclic	KHLWLW	Xaa_1-His-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
28 – cyclic	KSLWLW	Xaa_1-Ser-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Lys Xaa_2 = D-Leu
29 – cyclic	RRLWLW	Xaa_1-Arg-Xaa_2-Trp-Xaa_2-Trp	Xaa_1 = D-Arg Xaa_2 = D-Leu
30 – cyclic	QRUUUFWR	Glu-Xaa_1-Xaa_2-Xaa_3- Xaa_2-Xaa_3-Trp-Xaa_1	Xaa_1 = D-Arg Xaa_2 = L-hydrophobic Xaa_3 = D-hydrophobic Xaa_4 = D-Phe

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SEQ ID NO:	Shorthand	Sequence Listing	Xaa Definition
31 – cyclic	QRUUUWWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$- Xaa$_2$-Xaa$_3$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-hydrophobic Xaa$_4$ = D-Trp
32 – cyclic	QRUUUAWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$- Xaa$_2$-Xaa$_3$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-hydrophobic Xaa$_4$ = D-Ala
33 – cyclic	QRUUULWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$- Xaa$_2$-Xaa$_3$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-hydrophobic Xaa$_4$ = D-Leu
34 – cyclic	QRUUFWWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$- Phe-Xaa$_4$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-hydrophobic Xaa$_4$ = D-Trp
35 – cyclic	QRUWLWWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$-Leu-Xaa$_3$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-Trp
36 – cyclic	QRWWLWWR	Glu-Xaa$_1$-Trp-Xaa$_2$-Leu-Xaa$_2$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = D-Trp
37 – cyclic	QRUUWWWR	Glu-Xaa$_1$-Xaa$_2$-Xaa$_3$-Trp-Xaa$_4$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = L-hydrophobic Xaa$_3$ = D-hydrophobic Xaa$_4$ = D-Trp
38 – cyclic	QRVWLWWR	Glu-Xaa$_1$-Val-Xaa$_2$-Leu-Xaa$_2$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = D-Trp
39 – cyclic	QRFWLWWR	Glu-Xaa$_1$-Phe-Xaa$_2$-Leu-Xaa$_2$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = D-Trp
142 – cyclic	QRWWLWWR	Glu-Xaa$_1$-Trp-Xaa$_2$-Leu-Xaa$_2$-Trp-Xaa$_1$	Xaa$_1$ = D-Arg Xaa$_2$ = D-Trp

Table 19: Activity of Peptides Containing D-,L-α-Amino Acids

Against *S. aureus* and *E. coli*

SEQ ID NO:	Shorthand Sequence[c]	*S. aureus* (MRSA)[a] MIC μg/ml	*E. coli*[b] MIC μg/ml	Hemolysis HD50 μg/ml
5 – cyclic	SKSWLWLW	10	100	30
6 – cyclic	THSWLWLW	100	100	100
7 – cyclic	RGDWLWLW	100	100	100
8 – cyclic	KQRWLWLW	6	80	45
9 – cyclic	KQRWLWLW	8	80	40
10 – cyclic	RQRWLWLW	18	90	25
11 – cyclic	KQKWLWLW	45	100	50
12 – cyclic	KSKWLWLW	5	40	100
13 – cyclic	SHKWLWLW	10	100	35
14 – cyclic	SKHWLWLW	15	100	20
15 – cyclic	SHHWLWLW	20	100	20
16 – cyclic	EKHWLWLW	100	100	)100
17 – cyclic	KKKWLWLW	8	70	50
18 – cyclic	RRKWLWLW	6	15	50
19 – cyclic	KRKWLWLW	10	40	50
20 – cyclic	RRRWLWLW	10	50	35
21 – cyclic	HKHWLWLW	12	15	25
22 – cyclic	KHKWLWLW	10	80	30
23 - linear	WKKKWLWLW	60		
24 - linear	KKKWLWLW	50		
25 - linear	Ac-KKKWLWLW-CONH2	80		
26 – cyclic	KKLWLW	10	17	80
27 – cyclic	KHLWLW	10	100	25
28 – cyclic	KSLWLW	75	100	90
29 – cyclic	RRLWLW	35	5	90
30 – cyclic	QRUUUFWR[d]			

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SEQ ID NO:	Shorthand Sequence[c]	*S. aureus* (MRSA)[a] MIC μg/ml	*E. coli*[b] MIC μg/ml	Hemolysis HD50 μg/ml
31 – cyclic	QRUUUWWR[d]			
32 – cyclic	QRUUUAWR		37	
33 – cyclic	QRUUULWR			
34 – cyclic	QRUUFWWR[d]	2.5 - 3.8		
35 – cyclic	QRUWLWWR[d]			
36 – cyclic	QRWWLWWR[d]	2.5 - 3.8		
37 – cyclic	QRUUWWWR	2.5 - 3.8		
38 – cyclic	QRVWLWWR	2.5 - 3.8		
39 – cyclic	QRFWLWWR	2.5 - 3.8		
142 – cyclic	QRWWLWWR	2.5 - 3.8		

a. ATCC 33591. b. JM109(DE3). c. U is a hydrophobic amino acid selected from the group consisting of alanine, leucine, valine, phenylalanine and tryptophan. d. Activity against *Bacillus subtilis* at about 1.7 to 2.5 μg/ml. Peptide mixtures SEQ ID NO:30-37 are complex peptide mixtures and their concentrations for the anti-bacterial assay could not be specifically determined. The MIC values for these sequences may differ by up to five-fold.

The role of polar side chains in bioactivity and membrane selectivity may be evaluated upon examination of the antibacterial activities listed in Table 19. Cyclic peptides with SEQ ID NO:5 and 6 each bear one basic residue between two serine residues, or between a serine and a threonine residue, respectively. Peptide SEQ ID NO:5 displayed good activity against *S. aureus* MRSA, while substitution of lysine by histidine in peptide SEQ ID NO:6 decreases the activity. Cyclic peptides with SEQ ID NO:8-15 each possess two basic amino acids and one neutral, polar amino acid. These cyclic peptides varied in activity and red blood cell hemolysis. A single glutamic acid substitution affected activity as indicated by the difference in activity against *S. aureus* MRSA bacteria between peptide SEQ ID NOs:14 and 16. Increasing the number of basic residues from two to three in peptide SEQ ID NOs:17-22 provided significant activity against *S. aureus* MRSA. Peptides with SEQ ID NO:18 and SEQ ID NO:21 also exhibited activities against *E. coli*. The *in vitro* antibacterial activities of hexameric peptides with SEQ ID NOs:26-29 also indicate that use of basic amino acids in the cyclic peptides may increase activity and improve

membrane selectivity. Peptide SEQ ID NO:26 , with two consecutive lysine residues, exhibits broad-spectrum activity and has low hemolytic properties. On the other hand, peptide SEQ ID NO:27 , which has a histidine instead of the lysine in peptide SEQ ID NO:26, retains high activity against *S. aureus* MRSA but not against *E. coli*. Substituting a lysine in peptide SEQ ID NO:26 with serine yields a less active peptide SEQ ID NO:28. However, peptide SEQ ID NO:29, which possesses two arginine residues, displays potent and selective activity against *E. coli* with low levels of hemolysis. The spectrum of activity and membrane selectivity observed with the above peptides indicates that single amino acid substitutions can be utilized to influence activity and target cell selectivity.

Results for further testing of gram-negative and gram-positive bacteria are shown in Tables 20 and 21 respectively, along with control assays using FDA approved antibiotics. Underlining indicates that amino acid is a D-amino acid residue and brackets indicate that the peptides are circular.

Table 20: Gram Negative Bacteria

Peptide Sequence	*E. coli* (K12) MHII broth	*E. coli* (EL744) K12 (tolC::kan) MHII broth	*H. influenzae* ATCC 49247 HTM
	MIC (ug/mL)	MIC (ug/mL)	MIC (ug/mL)
[KKLWLW]-HCl	>128	32	>128
[KSKWLWLW]-HCl	>128	>128	32
[RRLWLW]-HCl	16	8	8-16
[RRKWLWLW]-HCl	32	16	32-64
Kanamycin	4	>128	4
Norfloxacin	0.06	0.016	0.06
penicillin G	32	16	4
Vancomycin	128	128	128

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Table 21: Gram-Positive Bacteria

Peptide Sequence	*E. faecalis* ATCC 51299 BHI broth	*E. faecium* SP180 BHI broth	*S. aureus* ATCC 33592 MHII broth
	MIC (ug/mL)	MIC (ug/mL)	MIC (ug/mL)
[KKLWLW]-HCl	>128	16	32
[KSKWLWLW]-HCl	8	4	4
[RRLWLW]-HCl	32-64	8-16	32
[RRKWLWLW]-HCl	8-16	4	8
Kanamycin	>128	>128	>128
Norfloxacin	2	32	0.5
Penicillin G	0.5	128	64
Vancomycin	32	>128	0.5

The selectivity of different types of cyclic peptides of the invention is further illustrated in Table 22, where underlining indicates which amino acids have D-chirality, brackets identify cyclic peptides and a number in parentheses indicates the SEQ ID NO:. These peptides were tested against methicillin-resistant *Staphylococcus aureus* (MRSA)(ATCC 33591) and vancomycin-resistant *Enterococcus faecalis* (VRE)(ATCC 51575) and for hemolytic activity against mammalian red blood cells.

Table 22: *In Vitro* Activity Against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococcus faecalis* (VRE)

Peptide sequence	MRSA	VRE	RBC Hemolysis (HD_{50})	Media Used
Control Antibiotics				
KANAMYCIN	150	>600	>600	b,c
MELLITIN	6.3	6.3	10	b,c
NORFLOXACIN	<2.8	<2.8	>360	b,c
VANCOMYCIN	<1.8	>240	>240	b,c
Group-I octamers				
KFWLWLWF (40)	33	>44	>44	a,a
KLWLWLWL (41)	12	25	30	b,c

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Peptide sequence	MRSA	VRE	RBC Hemolysis (HD_{50})	Media Used
Group-II octamers				
KSWLWLWF (42)	>26	>26	>26	a,a
KWWLWLWH (43)	13	17	>23	a,a
KWWLWLKW (44)	4	4	>58	A
Group-III octamers				
EKHWLWLW (16)	100	100	>100	a,a
HKHWLWLW (21)	12	16	25	a,a
KHKLALAL (45)	50	200	>200	b,c
KHKLALWL (46)	25	50	>200	b,c
KHKLFLAL (47)	3	13	>220	b,c
KHKLFLFL (48)	7	13	>220	b,c
KHKLFLWL (49)	12	25	200	b,c
KHKLWLWL (50)	9	18	150	b,c
KHKWLWLW (22)	10	10	30	a,a
KKKFLFLF (51)	18	75	>300	b,c
KKKWLWLW (17)	10	40	45	a,a
KKKWLWLW-HCl (17)	6	6	75	b,c
KQRWLWLW (9)	12	12	40	a,a
KQRWLWLW-HCl (8)	8	8	70	b,c
KRKLFLFL (52)	11	22	>180	b,c
KRKWLWLW (19)	15	8	50	a,a
KSKLALAL (53)	35	>280	>280	b,c
KSKLFLFL (54)	50	200	>200	b,c
KSKLFLFL (55)	200	50	>200	b,b
KSKLGLGL (56)	>240	>240	>240	b,c
KSKLILIL (57)	200	>200	>200	b,c
KSKLILIL (58)	>200	50	>200	b,b
KSKLNleLNleL (59)	220	>200	>220	b,c
KSKLVLVL (60)	>220	>220	>220	b,c
KSKLWLWL (61)	15	15	125	b,c
KSKLYLYL (62)	100	200	>200	b,c
KSKWLWLW (12)	5	15	100	a,a
KSKWLWLW-HCl (12)	9	9	60	b,c
KSOLWLWL (63)	12	25	150	b,c
KTKLALAL (64)	80	>160	>160	b,c
KTKLFLFL (65)	60	>240	>240	b,c
KTKLWLWL (66)	18	18	150	b,c
KWWLWLKS (67)	7	10	>153	b,b
QRFWLWWR (68)	7.5	3.8	60	b,b
QRLWLWWR (69)	6.3	6.3	35	b,b

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Peptide sequence	MRSA	VRE	RBC Hemolysis (HD_{50})	Media Used
QRVWLWWR (70)	12.5	6.3	30	b,b
QRWWLWWR (71)	6.3	6.3	35	b,b
RGDWLWLW (7)	100	100	100	a
RQRWLWLW (10)	10	10	25	a
RRKWLWLW (18)	5	5	50	a
RRKWLWLW-HCl (18)	9	9	70	b,c
SHHWLWLW (15)	30	50	20	a
SHHWLWLW-HCl (15)	6	6	40	b,c
SHKWLWLW (13)	10	40	35	a,a
SKHWLWLW (14)	15	30	20	a,a
SKSWLWLW (5)	20	20	30	a,a
THSWLWLW (6)	100	100	100	a,a
Group-IV octamers				
KHKHFLWL (72)	8	16	200	b,c
KHKHWLWL (73)	18	18	150	b,c
KRWLWLKH (74)	5	7	>130	a,a
KRWLWLKS (75)	9	22	>48	a,a
KSKKFLFL (76)	5	10	100	b,c
KSKKWLWL (77)	13	27	>220	b,c
KSSKALAL (78)	>240	>240	>240	b,c
KSSKFLFL (79)	43	43	>200	b,c
KSSKVLVL (80)	22	180	>180	b,c
KSSKWLWL (82)	27	27	>220	b,c
KSSKYLYL (83)	85	170	>340	b,c
KSWLWLRS (84)	9	12	>62	a
Group-V octamers				
KHKHKWLW (85)	6	6	150	b,c
KHQHKLWL (86)	100	100	>200	b,c
KHQHKWLW (87)	6	12	200	b,c
KHSHKWLW (88)	6	6	200	b,c
KNQNKFWF (89)	17	70	280	b,c
KNQNKLWL (90)	>200	>200	>200	b,c
KSKSKWLW (91)	6	12	200	b,c
KSQSKWLW (92)	9	18	195	b,c
KSSSKWLW (93)	6	6	175	b,c
KYQYKNWN (94)	>200	>200	>200	b,c
Group-VI octamers				
KAQAKAWA (95)	>200	>200	>200	b,c

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Peptide sequence	MRSA	VRE	RBC Hemolysis (HD_{50})	Media Used
KAQNKAWN (96)	>200	>200	>200	b,c
KAQFKAWF (97)	100	100	>200	b,c
KFQFKNWN (98)	>200	>200	>280	b,c
KFWLWLHW (99)	21	21	>25	a
KFWLWLKF (100)	11	10	>78	a
KLKLKLKL (3)	>200	>200	>200	b,c
KRKWLHLW (101)	40	40	100	a
KRQFKFWF (102)	12	12	>200	b,c
KSKLHLHL (103)	>320	>320	>320	b,c
KSKLQLQL (104)	>200	>200	>200	b,c
KSKLRLRL (105)	>240	>240	>240	b,c
KSKLRLRL (106)	>400	>400	>400	b,c
KWKWSWLW (107)	12	12	150	b,c
KYQYKNWN (108)	>200	>200	>200	b,c
RRKWLELW (109)	80	100	100	a
RRKWLHLW (110)	25	40	100	a
RRKWLKLW (111)	12	80	100	a
RRKWLSLW (112)	12	50	100	a
Group-II hexamers				
KHLWLW (27)	10	40	25	a
KKLWLW (26)	10	40	80	a
KRVWAI (113)	>200	>200	>200	b,c
KSLWLW (28)	100	100	90	b,c
KWILAR (114)	50	>200	>200	b,c
RRWLWL (115)	40	100	90	a,a
KWQWLW (116)	12	12	>16	a,a
Group-III hexamers				
KEKWLW (117)	101	91	>121	a,a
KEQWLW (118)	>115	>115	>115	a,a
KHKWLW (119)	12	60	>115	a,a
KHQWLW (120)	43	57	115.0	a,a
KKKWLW (121)	23	51	>111	a,a
KKQWLW (122)	27	81	>215	a,a
KNQWLW (123)	>74	>74	>74	a,a
KQQWLW (124)	>35	>35	>35	a,a
KRKWLW (125)	9	33	>174	a,a
KSKWLW (126)	19	38	>65	a,a
KSYVWS (127)	>200	>200	>200	b,c

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Peptide sequence	MRSA	VRE	RBC Hemolysis (HD_{50})	Media Used
Group-VI hexamers				
KFKWLW (128)	14	43	>61	a,a
KFQWLW (129)	25	10	>81	a,a
KHQSRT (130)	>200	>200	>200	b,c
KHQSRT (131)	>200	>200	>200	a,a
KSIRIT (132)	>200	>200	175	b,c

All peptides tested were TFA salts except where noted. Media "a" was anti-microbial media-3 (AM-3). Media "b" was cation adjusted Meuller Hinton broth (MHBII). Media "c" was Brain Heart Infusion (BHI).

In another series of experiments (Table 23), the activity and selectivity of cyclic peptides of the invention was tested against a larger variety of bacterial species: Vancomycin resistant *Enterococcus faecalis* (VRE, ATCC 51575); methicillin resistant *Staphylococcus aureus* (ATCC 33591, MRSA); *E. coli*: JM109 (DE3) *Bacillus cereus* (ATCC11778); and *Streptococcus pneumoniae* (ATCC 6301). Murine red blood cells were used for the hemolysis assays as described in Example 1.

Table 23: Minimum Inhibitory Concentrations (MIC) in mg/ml

Peptide	VRE	MRSA	E. coli	B. cereus	S. pneum.	Hemolysis HD_{50} mg/ml
Group-I octamer						
KLWLWLWL (3)	80	ppt	100	50	50	20
Group-II octamer						
KELWLWLW (143)	100	100		>140	100	
Group-III octamer						
KSKWLWLW (12)	15	5	40	<3	10	100
RRKWLWLW (18)	5	5	15	5	5	50
RQRWLWLW (10)	10	10	100	<1	4	25
SHKWLWLW (13)	40	10	100	12		35
KKKWLWLW (17)	40	10	65	5	10	45
RRRWLWLW (133)		10	35	10	5	35
KHKWLWLW (22)	10	10	80	8	8	30
KQRWLWLW (9)	12	12	80	3	4	40
HKHWLWLW (21)	16	12	15	4	19	25
SKHWLWLW (14)	30	15	100	6	5	20
KRKWLWLW (19)	8	15	60	3	5	50

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Peptide	VRE	MRSA	E. coli	B. cereus	S. pneum.	Hemolysis HD_{50} mg/ml
SKSWLWLW (5)	20	20	100	>30	30	30
KQKWLWLW (11)	20	20	100	7	8	50
SHHWLWLW (15)	50	30	100	11	40	20
THSWLWLW (6)	100	100	100	>280	100	100
RGDWLWLW (7)	100	100	100	>280	70	100
EKHWLWLW (16)	100	100	100	85	50	>100
Group-VI octamer						
RRKWLKLW (111)	80	12	6	23	25	100
RRKWLSLW (112)	50	12	12	13	12	100
RRKWLHLW (110)	40	25	15	20	20	100
KRKWLHLW (101)	40	40	12	80	80	100
RRKWLELW (109)	100	80	40	195	100	100
Linearized octamers						
H-WKKKWLWL-OH (144)	100	60		340	100	
H-KKKWLWLW-NH2 (134)	100	30	3	19	80	100
Ac-KKKWLWLW-NH2 (25)	100	100	20	75	80	100
H-RRKWLWLW-NH2 (135)	100	20	4	19	40	100
Ac-RRKWLWLW-NH2 (136)	100	70	6	22	40	100
H-LWRRKWLW-NH2 (137)	40	5	4	9	20	100
Ac-LWRRKWLW-NH2 (138)	100	15	7	20	20	100
Backbone modified octamers						
c[RRKW^{Me}LW^{Me}LW] (139)	30	5	5	5	10	100
c[KQRW^{Me}LW^{Me}LW] (140)	100	10		8	10	
Group-II hexamer						
KKLWLW (26)	40	10	17	>10	30	80
KHLWLW (27)	40	10	100	5	5	25
RKLWLW (141)	25	25		13	20	
RRWLWL (115)	100	40	5	24	12	90
KSLWLW (28)	100	100	100	15	20	90

EXAMPLE 7

Effect of Plasma Proteins and Proteolysis On Cyclic Peptides

The effect of plasma proteins on the availability and stability of cyclic peptides *in vitro* was also examined. Activity remained unchanged in the presence of large amounts (up to 50% v/v) of fetal bovine serum (FBS) in the

culture media. However, most peptides examined have a reduced level of hemolytic activity in the presence of 5-10% FBS in the assay mixture, compared to analogous assays in the absence of FBS. For example, in the presence of 10% FBS, peptide SEQ ID NO:8 displays five-fold reduction in hemolytic activity (from HD_{50} = 10 to 50 μg/ml). Hence, less hemolysis occurred when more fetal bovine serum was present.

Cyclic peptides having SEQ ID NO:13, 18, 26, and 29 were also assayed for proteolytic susceptibility. The peptides have an abiotic structure and conformational preferences, and were stable in the presence of trypsin, α-chymotrypsin, subtilisin, and blood plasma. No significant peptide degradation was observed in chromatograms obtained from RP-HPLC over a 24 h time period, whereas control linear L-α-amino acid peptides were degraded in less than 10 min under similar reaction conditions and within four hours when placed in murine blood plasma.

EXAMPLE 8

Membrane Depolarization Activity

Although not intending to be bound by any particular theory or mechanism of action, it is believed that one mechanism by which the present cyclic peptides may kill or affect target cells is by membrane depolarization. Another mechanism by which the cyclic peptides may kill or affect cells is through a receptor that recognizes the cyclic peptides as ligands. *See* Friederich et al., *Antimicrob. Agents Chemother.* 44, 2086-2092(2000); Amsterdam, D. in *Antibiotics in Laboratory Medicine,* 3rd ed. (ed. Lorian, V.) 53-105 (Baltimore, Maryland, USA, 1991), although this mode of action for the cyclic peptides in membranes is less likely for several reasons.

The structural diversity of cyclic peptides with activity is illustrated by enantiomeric peptides having SEQ ID NO:8 (cyclo-[D-Lys-Gln-D-Arg-Trp-D-Leu-Trp-D-Leu-Trp]) and SEQ ID NO:9 (cyclo-[Lys-D-Gln-Arg-D-Trp-Leu-D-Trp-Leu-D-Trp]). These two cyclic peptides have similar *in vitro* activities despite the differences in chirality of these peptides at each position.

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In this Example, cyclic peptides were tested to determine cellular depolarization activity. The fluorescence of the cyanine membrane dye 3,3'-dipropylthiadicarbocyanide is sensitive to changes in bacterial membrane potential, and was employed in this Example to follow the effects of peptide SEQ ID NO:8, 18 and 26 on membrane depolarization of live *S. aureus* (ATCC 25923). *See,* Sims et al., *Biochemistry* 13, 3315-3330 (1974); Waggoner, *Annu. Rev. Biophys. Bioeng.* 8, 847-868 (1979); Loew, *Adv. Chem. Ser.* 235 (Biomembrane Electrochemistry), 151-173 (1994). In each case, the exposure of dye-saturated live *S. aureus* to cyclic peptides at various concentrations (0.1 to 1 x MIC, minimum inhibitory concentration) led to fast and complete membrane depolarization. Culture samples taken during the course of these experiments correlate membrane depolarization with cell death. Substantially no living bacteria could be detected after five minutes exposure to MIC concentrations of peptides, whereas at 0.1xMIC concentrations a significant population of live bacteria was still present.

Biophysical analyses performed in synthetic lipid membranes also support a membrane permeation mode of action. Studies support the idea, for example, that eight residue cyclic D, L-α-peptides can likely form different types of supramolecular structures depending on the amino acid composition and sequence of the cyclic peptide. For example, the peptide SEQ ID NO:2 (cyclo[-Gln-D-Leu-(Trp-D-Leu)$_3$]) facilitates only the transport of analytes that are smaller than its internal diameter across liposome membranes and according to ATR-FTIR analysis in DMPC multibilayes, assembles into a tube-like structure that is oriented perpendicular to the membrane plane. Therefore, the single nanotube through-pore mechanism it is a likely mode of function for this peptide (see Figure 2a). On the other hand, the homologous peptide SEQ ID NO:3 (cyclo[-Lys-D-Leu-(Trp-D-Leu)$_3$]), having a polar charged side chain likely forms a different supramolecular structure with an opening that is larger than the internal tube diameter because it facilitates transport of larger molecules of up to approximately 10,000 MW across membranes. Furthermore, the supramolecular structure formed from peptides having SEQ ID NO:3 (cyclo[-Lys-D-Leu-(Trp-

D-Leu)$_3$]) maintains an orientation in synthetic membranes that is perpendicular to the membrane plane according to ATR-FTIR analysis. These data support the conclusion that the peptide likely forms a barrel-stave type supramolecular structure in which multiple upright nanotubes bundle to form a larger pore opening (Figure 2b).

In comparison, peptides like SEQ ID NO:11 (cyclo[-(D-Lys-Gln-D-Lys-(Trp-D-Leu)$_2$-Trp-], SEQ ID NO:8 (cyclo[-(D-Lys-Gln-D-Arg-(Trp-D-Leu)$_2$-Trp-], SEQ ID NO:17 (cyclo[-(D-Lys-Lys-D-Lys-(Trp-D-Leu)$_2$-Trp-], and SEQ ID NO:18 (cyclo[-(D-Arg-Arg-D-Lys-(Trp-D-Leu)$_2$-Trp-] which possess a hydrophilic face of three contiguous residues, not only mediate the transport of molecules larger than the internal peptide diameter (Figure 3), but also appear to adopt orientations that are approximately parallel to the plane of the membrane structure. The ATR FT-IR spectroscopy of cyclic peptide SEQ ID NOs:8, 17 and 18 in synthetic lipid membranes reveal amide-I and amide-II bands that are characteristic of tightly hydrogen bonded β-sheet-like structures (data not shown). The observed amide-A (NH stretch) frequencies support a tight network of intersubunit backbone hydrogen bonding. Quantitative measurements in oriented DMPC lipid multibilayers indicate that self-assembled peptide nanotubes are oriented at a 70±5° tilt angle from the membrane normal. These observations suggest a carpet-like mode of membrane permeation (Figure 2). Increasing the hydrophilicity of the cyclic peptide, thus appears to favor a "surface-seeking" orientation over a "pore-forming" orientation.

Table 23 ATR-FTIR data and orientation of cyclic peptides in oriented DMPC lipid multibilayers.

Cyclic Peptide SEQ ID NO:	Frequency (cm^{-1})			Peptide		Lipid		ΔTilt[d]
	Amide-I	Amide-II	Amide-A	[A‖ / A^][a]	Tilt[c]	[A‖ / A^][b]	Tilt[c]	
8 [KQRWLWLW]	1629	1538	3283	1.16	70°	1.16	29°	41°
17 [KKKWLWLW]	1629	1537	3281	1.13	71°	1.17	29°	42°
18: [RRKWLWLW]	1628	1538	3280	1.34	65°	1.15	29°	36°

[a] The dichroic ratio of the amide-I intensity with parallel polarized incident light to the band intensity with the perpendicular polarized light.
[b] The dichroic ratio of the antisymmetric CH_2 stretches of DMPC lipid.
[c] Tilts refer to the angle of the molecular axis with respect to the surface normal.
[d] Difference between the angles of the peptide tube axis and the lipid hydrocarbon chain. Lipid and peptide nanotube tilt angles are calculated according to methods detailed in H.-S. Kim et al., 120 J. Am. Chem. Soc.,4417-24 (1998). DMPC: dimyristoyl phosphatidylcholine. Data are average of two samples with errors <±2°.

Further evidence for cellular membrane activity was provided by electron micrographs of bacteria treated with cyclic peptides of the invention. Bacteria (*S. aureus*, ATCC 25923) were treated with cyclic peptide cyclo[KSKWLWLW] for 120 minutes at room temperature and then prepared for thin section electron microscopy by standard procedures. Figures 6-8 illustrate the membrane effects caused by cyclic peptides of the invention as observed using the electron microscope. Figure 6 provides a thin section electron microscopy image of untreated *S. aureus* (ATCC 25923) displaying a normal intact membrane. Figures 7 and 8 provide thin section electron microscopy images of *S. aureus* (ATCC 25923) after exposure to 2XMIC concentrations of cyclo[KSKWLWLW]. These images provide direct visualization of a membrane mode of action. Arrows denote abnormal membrane structures caused by the peptide action.

Evidence thus indicates that targeted cytotoxicity is based at least in part on membrane permeation, depolarization, and/or lysis. Such evidence includes the observation that the cyclic peptides act very quickly to kill microbes, that diverse cyclic peptide structures described herein show anti-cancer and anti-microbial activity, that the cyclic peptides can depolarize microbial membranes, that attenuated total reflectance (ATR) FT-IR spectroscopy studies are consistent with a membrane permeation mechanism of action rather than a receptor/ligand-mediated binding/inhibition mechanism, and that electron microscopy reveals an effect of the cyclic peptides of the invention on membrane structure.

EXAMPLE 9

Cyclic β-Peptides Self-Assemble to Form Transmembrane Ion Channels

While not intending to be bound by any particular theory or mechanism of action, the ion transport properties of tetrapeptides cyclo[(β-Trp)$_4$] and cyclo[(β-Trp-β-Leu)$_2$], synthesized via established protocols, were studied in liposome-based assays and by single channel conductance measurements in planar lipid membranes. The observed conductance in 500 mM KCl with peptide concentrations of about 30 mM in the subphase is 56 pS, corresponding

to the rates of channel mediated K^+ transport of 1.9×10^7 ions s^{-1} for both tetrapeptides. Such a transport speed is more than twice that of gramicidin A under similar conditions. FT-IR studies in lipid membranes were also undertaken that provided evidence of transmembrane channels formed by these peptides. Peptide preparations displayed all expected peptide IR signals including amide I, amide II and N-H bands. The observed amide N-H stretching bands at 3289 cm^{-1} and 3297 cm^{-1} indicate the existence of tight backbone hydrogen-bonding networks with an average inter-subunit distance of 4.8 Å that are consistent with solid state IR data on cyclic D, L-α-peptides. Therefore, cyclic β-peptides can also form transmembrane ion channels.

EXAMPLE 10

Cyclic Peptide Selectivity *In Vivo*

Initial toxicology studies in mice were conducted to evaluate various routes of drug administration, maximum tolerable dose, and blood and tissue toxicity.

Procedures

Peptides were tested for toxicity *in vivo* using procedures similar to those described previously. *See* V. Lorian, Antibiotics in Laboratory Medicine, Williams and Wilkins, Baltimore 1991. Mice (male Balb-C) in groups of 4-8 were given via IV, IP or SQ single bolus doses of peptides and monitored for 14 days. Toxicity of sub-lethal doses was assessed based on the behavior and appearance of the mice after peptide administration compared to control mice receiving only vehicle. Signs of acute toxicity included lack of activity, red feet and tail, faster breathing. Death was defined as the end point for lethal doses of peptides.

Pathology effects of peptides cyclo[RRKWLWLW]-HCl and cyclo-[KQRWLWLW]-HCl were accessed with Balb-C (male 20-25 g). Peptides were administered IP at a lethal dose of 75 mg/kg in 9% sucrose, along with control

mice that received vehicle alone. After 50-60 min (in case of cyclo[KQRWLWLW]-HCl), and on the next day (in case of cyclo[RRKWLWLW]-HCl) mice were sacrificed and analyzed. Pathology studies included blood cell count, and histology examination of different tissues and organs. Toxicity of multiple dosing in mice (male CD-1,Charles River labs, 20-25 g) was also tested with peptide cyclo[KSKWLWLW]-HCl. A regiment of 200 mg/kg per day of the peptide solution in 9% sucrose for ten consecutive days was administered to 3 mice IP, along with 2 control mice that received vehicle alone. On day eleven, mice were sacrificed and analyzed. Pathology studies included blood cell count, and histology examination of different tissues and organs.

Peptides of the invention were tested in vivo in mice to evaluate protection against bacterial infection using procedures similar to those described in N. Frimodt-M ller et al., *The Mouse Peritonitis/Sepsis Model*, in Zak et al. (eds.) HANDBOOK OF ANIMAL MODELS OF INFECTION 127-37 (Academic Press 1999). Male Balb-C mice (6 weeks old, approximately 20 g) were used in the study. *Staphylococcus aureus* MRSA bacteria (ATCC 33591) were grown at 37 °C with agitation for 12 hours to a stationary phase. Cells were collected by centrifugation, washed twice with saline and resuspended to an $O.D._{650}$ of 1.4. This suspension was diluted ten times with saline and resuspended to a concentration of $3\text{-}5x10^7$cfu/ml. For each peptide five groups of eight mice per group were infected I.P. with 0.5 ml of the above *Staphylococcus aureus* MRSA preparation (lethal dose). Forty-five minutes to an hour after infection, each group was treated with a different dose of peptide and a control group received vehicle only. Mice were monitored for fourteen days; death was defined as the end point. Median protecting doses (PD_{50}) were calculated following the Reed and Muench method. *See* V. Lorian, Antibiotics in Laboratory Medicine, Williams and Wilkins, Baltimore 1991. For IV and SQ models peptide was administered immediately after IP infection.

Pharmacokinetic analyses were performed using methods similar to those described previously. *See* V. Lorian, Antibiotics in Laboratory Medicine,

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Williams and Wilkins, Baltimore 1991; W. A. Ritschel, G. L. Kearns Handbook of Basic Pharmacokinetics, American Pharmaceutical Assoc. 5th edition, Washington, DC 1999. For pharmacokinetics studies with c[RRKWLWLW]HCl by IV injection, a solution of peptide in 9% sucrose (1 mg/mL) was injected IV into the tail vein of Balb/C mice at a dose of 3.6 mg/kg. Blood was collected immediately from one group of mice (3 mice per group) by bleeding the tail, separately from each mouse (50-100 uL per mouse). Blood was also collected from other groups of mice at times 20, 40, 70, 90, 120, 180, and 260 min after injection. Plasma from each blood sample was separated immediately after collection by spinning down red blood cells over a period of 5 min at 4000 rev./min. The plasma was diluted with an equal volume of saline and refrigerated until analysis. Storage of samples under these conditions over a period of 1 month did not change the concentration of peptide. For pharmacokinetics studies with c[RRKWLWLW]HCl by IP injection, solutions of peptide in 9% sucrose (8.1 mg/mL) were injected IP into Balb/C mice at a dose of 100 mg/kg. Blood was collected immediately from one group of mice (3 mice per group) by bleeding the tail, separately from each mouse (50-100 uL per mouse). Blood was also collected from other groups of mice at times 0.5, 1, 2, 4, 6, 10, 15 h after injection. Plasma from each blood sample was separated immediately after collection by spinning down red blood cells over a period of 5 min at 4000 rev./min. Plasma was then diluted with equal volume of saline and refrigerated until analysis. Storage of samples under these conditions over a period of 1 month did not change the concentration of peptide. Detection of cyclo[RRKWLWLW]-HCl in plasma by HPLC was accomplished as follows. Samples were diluted with saline plasma (50-100 uL), added to an equal volume of eluent A (0.1 % HCl in 99% H_2O/1% ACN (v/v)) (150-300 uL), vortexed, and partial precipitation was removed by centrifugation. The clear solution was injected into an HPLC and the peptide was detected at 280 nm, in the 8-10 min interval, using a gradient of eluent A (0.1 % HCl in 99% H_2O/1% ACN (v/v)) and eluent B (0.07 % HCl in 10% H_2O/90% ACN (v/v)) using a flow rate of 1.5

ml/min. The following gradient was used: 30 to 30 % B (5 min), followed by 30 to 37% B (5 min), followed by 37 to 40% B (12.5 min).

For pharmacokinetics studies with c[KSKWLWLW]HCl by IV injection, a solution of peptide in 9% sucrose (2 mg/mL) was injected IV into the tail vein of Balb/C mice at a dose of 5 mg/kg. Blood was then collected immediately from one group of mice (3 mice per group) by bleeding the tail, separately from each mouse (50-100 uL per mouse). Blood was also collected from other groups of mice at times 30, 60, 120, 230, and 300 min after injection. Plasma from each blood sample was separated immediately after collection by spinning down red blood cells over a period of 5 min at 4000 rev./min. Plasma was then diluted with an equal volume of saline and refrigerated until analysis. Storage of samples under these conditions over a period of 1 month did not change the concentration of peptide. For pharmacokinetics studies with c[KSKWLWLW]HCl by IV injection, a solution of peptide in 9% sucrose (9.8 mg/mL) was injected IP into Balb/C mice at a dose of 100 mg/kg. Blood was then collected immediately from one group of mice (3 mice per group) by bleeding the tail, separately from each mouse (50-100 uL per mouse). Blood was also collected from other groups of mice at times 0.5, 1, 2, 4, 6, 10 h after injection. Plasma from each blood sample was separated immediately after collection by spinning down red blood cells over a period of 5 min at 4000 rev./min. Plasma was then diluted with an equal volume of saline and refrigerated until analysis. Storage at these conditions over a period of 1 month did not change the concentration of peptide. Detection of c(KSKWLWLW) HCl in plasma by HPLC was accomplished as follows. Samples were diluted with saline plasma (50-100 uL), added to an equal volume of eluent A (0.1 % TFA in 96.5% H_2O/0.9% ACN/2.4% MeOH (v/v)), vortexed, and partial precipitation was removed by centrifugation. The clear solution was injected into HPLC and the peptide was detected at 280 nm, in the 18-20 min interval, using a gradient of eluent A (0.1 % TFA in 96.5% H_2O/0.9% ACN/2.4% MeOH (v/v)) and eluent B (0.05 % TFA in 8% H_2O/72% ACN/20% MeOH (v/v)) using a flow rate of 1.5

ml/min. The following gradient was used: 0 to 0 % B (5 min), followed by 0 to 100% B (25 min).

The area of HPLC peaks corresponding to the peptide in analytical plasma runs was correlated to those of the calibration injections with known amounts of peptide, and the determined concentration was plotted against the time of blood collection. In the first approximation the curve obtained from IV injection fits to the first order kinetic equation $C_t = C_0 * e^{-Kel*t}$ (equation 1), indicating a single compartment model. *See* V. Lorian, Antibiotics in Laboratory Medicine, Williams and Wilkins, Baltimore 1991; W. A. Ritschel, G. L. Kearns Handbook of Basic Pharmacokinetics, American Pharmaceutical Assoc. 5th edition, Washington, DC 1999. The best fit of equation (1) to the experimental points was calculated using SigmaPlot. The following parameters were calculated from the best fit of IV injection curve: AUC_{IV} (µg*h/mL) (area under the curve for IV injections); C_0 (µg/mL) (concentration of peptide in blood at time zero); K_{el} (1/min) (elimination rate constant); $T_{1/2}$ (min) (half life time (time when $C = C_0/2$); $T_{1/2} = (\ln 2)/K_{el}$); V (L/kg) (volume of distribution, $V = D/C_0$, where D is the dose of injected peptide expressed in mg/kg); CL (mL/min) (total clearance of peptide was calculated in two ways and averaged); $CL = Dose/AUC_{IV}$, where Dose is total dose of peptide received by mouse expressed in µg; and, $CL = K_{el} * V * m$, where m is mass of the mouse expressed in g. The equation $F = 100\%*(AUC_{IP} * D_{IV})/(AUC_{IV} * D_{IP})$ (equation 2) was used to determine bioavailability (F) of peptide injected via IP route where AUC_{IP} is area under the concentration vs. time curve from IP injections, D_{IV} and D_{IP} are doses of peptide administered via IV and IP routes respectively, expressed in mg/kg.

Results

In an initial dosage study, two mice in each dose group were injected with a bolus intravenous (IV), intraperitoneal (IP), or subcutaneous (SQ) doses of peptide SEQ ID NO:8, 18, and 26. For these studies, two control animals separately received vehicle only and no peptide. Studies with peptide SEQ ID

NO:8 indicated that bolus IV injections of 12 mg/kg caused signs of temporary (<10 min in duration) discomfort. In contrast, no signs of toxicity were observed at the maximum tested dose (12 mg/kg) with the IP route of administration. Peptide SEQ ID NO:26 was tolerated up to the maximum tested IP dose (50 mg/kg), with the highest dose causing signs of acute toxicity that were not observable after one hour. Signs of acute toxicity included any one of the following: lack of activity, red feet and tail, or faster breathing. Peptide SEQ ID NO:18 was tested up to 17.5 mg/kg IP and SQ doses without any apparent signs of toxicity. A regimen of 17.5 mg per day of peptide SEQ ID NO:18 for three consecutive days was administered to two mice IP and two mice SQ. Over four days, no apparent changes in the physical, social, and feeding activities were observed in these mice relative to control mice injected with vehicle without peptide. Moreover, hematology, necropsy and detailed microscopic examination of various tissue and organ samples performed on the fourth day after the initial injection showed normal blood and morphological profiles except for the sites of IP and SQ injections (K.G. Osborn, DVM, Ph.D., the Scripps Research Institute). These sites exhibited moderate subacute inflammation typical of IP and SQ drug administration.

Peptide SEQ ID NO:18 was tested for antibacterial activity *in vivo* by observing whether this peptide could protect mice from bacterial infection. Two groups of mice (4 mice per dose in each group) were infected intraperitoneally (left side) with a lethal dose of MRSA (ATCC 33591) ($2\text{-}5 \times 10^7$ cfu/mouse). To the first group of mice, peptide bolus doses of 0 (vehicle only), 10, 20, and 40 mg/kg were administered subcutaneously (SQ) in the upper neck compartment soon after MRSA injection. To the second group of mice, five bolus peptide doses each 0 (vehicle only), 2.5, and 5 mg/kg were administered intravenously (IV) in 10 hour intervals with the first series of doses administered soon after MRSA injection. All mice in the control groups that received vehicle without peptide (0 mg/kg doses) died within 48 hours. However, at 40 mg/kg bolus SQ dose and 2.5 x 5 mg/kg IV dosage regimen, 75% and 50% of mice survived, respectively, during the course of a fourteen-day study. There was some

variability observed in SQ and IV treatment data (possibly resulting from lower absorption of the peptide administered SQ and/or within the range of expected experimental errors in IV administration of drugs to mice, a small test animal), and the efficacy of the intraperitoneal (IP) route was also evaluated. Six mice were infected intraperitoneally (left side) with a lethal dose of MRSA ($2\text{-}5x10^7$ cfu/mouse). A 13 mg/kg bolus of the peptide was administered IP (right side) 45 min to an hour after MRSA injection. All mice in the control group that received vehicle without peptide died within 48 hours, while 67% of mice that received the peptide survived during the course of a seven-day study.

A larger study with peptides having SEQ ID NO: 8, 12, 17, 18, and 26 was also performed using groups of mice infected with lethal doses of MRSA (ATCC 33591) (IP left side). Each group of mice was treated with a bolus IP (right side) dose of peptide SEQ ID NO: 8, 12, 17, 18, or 26 at 45-60 min after initial infection and observed for up to 14 days. All mice in the control group that received vehicle without peptide died within 48 hours.

In each case, a single dose of the appropriate amount of peptide was sufficient to completely protect various groups of mice from MRSA infections (Table 24). Groups of mice were also infected with lethal doses of VREF (ATCC 51575) (IP left side). Each group of mice was treated with a bolus IP (right side) dose of peptide SEQ ID NO: 12, 17, or 18 at 45-60 min after initial infection and observed for 14 days. All mice in the control group that received vehicle without peptide died within 48 hours. In each case, a single dose of the appropriate amount of peptide was sufficient to completely protect various groups of mice from VFEF infections (Table 24).

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Table 24: *In vivo* Protection Doses (PD_{50}) and Lethal Doses (LD_{50}) of Cyclic Peptides

Sequence[a]	PD_{50} (μg/ml) MRSA[b]	VREF[c]	LD_{50}(IP) (μg/ml)	LD_{50}(IV) (μg/ml)
c[RRKWLWLW] (SEQ ID NO:18)	8	7	60	16
c[KQRWLWLW] (SEQ ID NO:8)	7	-	55	>12
c[KSKWLWLW] (SEQ ID NO:12)	20	5	>400	70
c[KKKWLWLW] (SEQ ID NO:17)	7	13	-	-
c[KKLWLW] (SEQ ID NO:26)	15	-	75	24

[a] Underlined letters represent D-residues. [b] MRSA (ATCC 33591). [c] VREF (ATCC 51575). Male Balb- C mice were used in groups of 8 (for MRSA) and 4 (for VREF and lethal doses).

All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents and publications.

The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the

absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality (for example, a culture or population) of such host cells, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are described within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

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WHAT IS CLAIMED:

1. A pharmaceutical composition for treating a cancer in an animal comprising a cyclic peptide having a sequence of from four to about sixteen alternating D- and L-α-amino acids in an amount effective to treat said infection caused by a target cancer in said animal.

2. The pharmaceutical composition of claim 1, wherein the cyclic peptide has a minimum inhibitory concentration that is effective to inhibit said cancer and that is less than about one half the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells *in vitro*.

3. The pharmaceutical composition of claim 1, wherein the cyclic peptide has a minimum inhibitory concentration that is effective to inhibit said cancer and that is less than about one fifth the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells *in vitro*.

4. The pharmaceutical composition of claim 1, wherein the cyclic peptide has a minimum inhibitory concentration that is effective to inhibit said cancer and that is less than about one tenth the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells *in vitro*.

5. The pharmaceutical composition of claim 1, wherein the cyclic peptide has a minimum inhibitory concentration that is effective to inhibit said cancer and that is less than about one twentieth the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells *in vitro*.

6. The pharmaceutical composition of claim 1, wherein the effective amount induces substantially no hemolysis of mammalian red blood cells *in vitro*.

7. The pharmaceutical composition of claim 1, wherein the effective amount is a single dosage.

8. The pharmaceutical composition of any one of claims 1, 2, 3, 4, and 5, wherein said effective amount of said peptide inhibits the growth of said cancer in an animal.

9. The pharmaceutical composition of claim 1, wherein the cyclic peptide forms a structure comprising two or more non-covalently associated cyclic peptide molecules.

10. The pharmaceutical composition of claim 1, wherein the cyclic peptide self-assembles into a supramolecular structure.

11. The pharmaceutical composition of claim 10, wherein the supramolecular structure comprises a nanotube, a barrel of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof.

12. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least one polar D- or L-amino acid.

13. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least two polar D- or L-amino acids.

14. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least three polar D- or L-amino acids.

15. The pharmaceutical composition of claim 1, wherein the cyclic peptide has four to six polar D- or L-amino acids.

16. The pharmaceutical composition of any one of claims 13, 14 and 15, wherein each polar D- or L-amino acid is adjacent to at least one other polar D- or L-amino acid.

17. The pharmaceutical composition of any one of claims 12, 13, 14 and 15, wherein at least one polar D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids.

18. The pharmaceutical composition of any one of claims 12, 13, 14 and 15, wherein each polar D- or L-amino acid is a D- or L-enantiomer of cysteine, homocysteine, serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, histidine, arginine, lysine, hydroxylysine or ornithine.

19. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least one ionizable D- or L-amino acid.

20. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least two ionizable D- or L-amino acids.

21. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least three ionizable D- or L-amino acids.

22. The pharmaceutical composition of claim 1, wherein the cyclic peptide has four to six ionizable D- or L-amino acids.

23. The pharmaceutical composition of any one of claims 20, 21 or 22, wherein each ionizable D- or L-amino acid is adjacent to at least one polar D- or L-amino acid.

24. The pharmaceutical composition of any one of claims 19, 20, 21 or 22, wherein at least one ionizable D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids.

25. The pharmaceutical composition of any one of claims 19, 20, 21, and 22, wherein each ionizable D- or L-amino acid is arginine, aspartic acid, glutamic acid, histidine, lysine, hydroxylysine or ornithine.

26. The pharmaceutical composition of claim 1, wherein the cyclic peptide has at least one nonpolar D- or L-amino acid residue.

27. The pharmaceutical composition of claim 1, wherein the cyclic peptide has two to fifteen nonpolar D- or L-amino acid residues.

28. The pharmaceutical composition of claim 26 or 27, wherein each nonpolar D- or L-amino acid residue is alanine, valine, isoleucine, leucine, methionine, norleucine, phenylalanine, tyrosine or tryptophan.

29. A pharmaceutical composition for treating a cancer in an animal comprising a pharmaceutically acceptable carrier and a cyclic peptide having a sequence of about six alternating D- and L-α-amino acids in an amount effective to treat or prevent said infection caused by a target cancer in said animal.

30. A pharmaceutical composition for treating a cancer in an animal comprising a pharmaceutically acceptable carrier and a cyclic peptide having a sequence of about eight alternating D- and L-α-amino acids in an amount effective to treat or prevent said cancer in said animal.

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31. The pharmaceutical composition of any one of claims 1, 29 or 30, wherein the cyclic peptide has an amino acid sequence of formula I:

$$\begin{bmatrix} (Y_1)_p\text{-}(X_1)_m\text{-}(Y_2)_p\text{-}(X_2)_p\text{-}(Y_3)_p\text{-}(X_3)_p\text{-}(Y_4)_p\text{-}(X_4)_p\text{-}(Y_5)_p\text{-}(X_5)_p \\ (X_{10})_p\text{-}(Y_{10})_p\text{-}(X_9)_p\text{-}(Y_9)_p\text{-}(X_8)_p\text{-}(Y_8)_p\text{-}(X_7)_p\text{-}(Y_7)_p\text{-}(X_6)_p\text{-}(Y_6)_p \end{bmatrix}$$

I

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid; and

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

32. The pharmaceutical composition of claim 1, 29 or 30, wherein the cyclic peptide has an amino acid sequence of formula II:

$$\begin{bmatrix} (D\text{-}X_1\text{ -}L\text{-}X_2)_m\text{ - }(D\text{-}Y_1\text{ - }L\text{-}Y_2)_p\text{- }(L\text{-}X_3\text{ - }D\text{-}X_4)_p\text{- }(L\text{-}Y_3\text{ - }D\text{-}Y_4)_p \\ (L\text{-}Y_8\text{ - }D\text{-}Y_7)_p\text{ - }(D\text{-}X_8\text{ -}L\text{-}X_7)_p\text{- }(D\text{-}Y_6\text{ - }L\text{-}Y_5)_p\text{ - }(L\text{-}X_6\text{ - }D\text{-}X_5)_p \end{bmatrix}$$

II

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, and Y_8 is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

33. The pharmaceutical composition any one of claims 1, 29 or 30, wherein the cyclic peptide has an amino acid sequence having formula III:

$$\left[(X_1)_p\text{-}(X_2)_p\text{-}(X_3)_p\text{-}(X_4)_m\text{-}(X_5)_p\text{-}(X_6)_p\text{-}(X_7)_p\text{-}(X_8)_p\text{-}(X_9)_p\text{-}(X_{10})_p\right]$$
$$\left[(Y_{10})_p\text{-}(Y_9)_p\text{-}(Y_8)_p\text{-}(Y_7)_p\text{-}(Y_6)_p\text{-}(Y_5)_p\text{-}(Y_4)_p\text{-}(Y_3)_p\text{-}(Y_2)_p\text{-}(Y_1)_p\right] \qquad \textbf{III}$$

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

34. The pharmaceutical composition any one of claims 1, 29 or 30, wherein the cyclic peptide has an amino acid sequence of formula IVa or IVb:

$$\text{D-}X_1 - \underbrace{(\text{L-}X_2 - \text{D-}X_3)_n - (\text{L-}Y_1 - \text{D-}Y_2)_m - \text{L-}Y_3} \qquad \text{IVa}$$

or

$$\text{L-}X_1 - \underbrace{(\text{D-}X_2 - \text{L-}X_3)_n - (\text{D-}Y_1 - \text{L-}Y_2)_m - \text{D-}Y_3} \qquad \text{IVb}$$

wherein:

n is an integer ranging from 0 to 4;

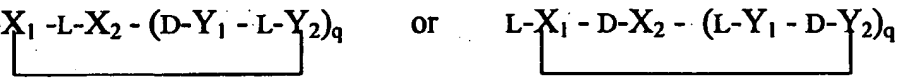
m is an integer ranging from 1 to 7;

X_1, X_2 and X_3 are each a separate a polar amino acid;

Y_1, Y_2 and Y_3 are each a separate nonpolar amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

35. The pharmaceutical composition of claim 1, 29 or 30, wherein the cyclic peptide has an amino acid sequence of formula Va or Vb:



Va Vb

wherein:

q is an integer ranging from 2 to 7;

X_1 and X_2 are separately polar amino acids;

Y_1 and Y_2 are separately nonpolar amino acids.

36. The composition of claim 1, wherein the composition comprises a mixture of two or more different cyclic peptides.

37. The pharmaceutical composition of claim 1, wherein the amino acid sequence comprises SEQ ID NO:8, 9, 12, 17, 18, 26, 29, 47-52, 61, 63, 67, 68, 72-77, 84, 85, 87-89, 91-93, 100, 102, 107, 111, 112, 119, 125, 139 or a mixture thereof.

38. The pharmaceutical composition of claim 1, wherein the amino acid sequence comprises SEQ ID NO: 72, 85, 91, 93, 125, 126, 133, 145, 146, 147, 148, 149, 151, 152, 155, 156, 157 or a mixture thereof.

39. A pharmaceutical composition for treating a cancer in an animal comprising a pharmaceutically acceptable carrier and a cyclic peptide having a homochiral sequence of from three to about ten β-amino acids,

wherein at least one β-amino acid has at least one polar side chain, in an amount effective to treat or prevent said infection caused by a target cancer in said animal.

40. The pharmaceutical composition of claim 39, wherein the cyclic peptide self-assembles into a supramolecular structure comprising a nanotube, a barrel of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof.

41. The pharmaceutical composition of claim 39, wherein the effective amount induces substantially no hemolysis of red blood cells *in vitro*.

42. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least one polar side chain and at least one non-polar side chain on adjacent β amino acids.

43. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least two polar side chains.

44. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least three polar side chains.

45. The pharmaceutical composition of claim 39, wherein the cyclic peptide has four to five polar side chains.

46. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least one ionizable side chain.

47. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least two ionizable side chains.

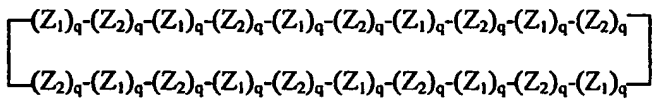
48. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least three ionizable side chains.

49. The pharmaceutical composition of claim 39, wherein the cyclic peptide has four to five ionizable side chains.

50. The pharmaceutical composition of claim 39, wherein the cyclic peptide has at least one nonpolar side chain.

51. The pharmaceutical composition of claim 39, wherein the cyclic peptide has two to nine nonpolar side chains.

52. The pharmaceutical composition of claim 39, wherein the cyclic β-peptide has an amino acid sequence of formula IV:



IV

wherein:

each q is separately an integer ranging from 0 to 7;

each Z_1, Z_3, Z_5, Z_7, Z_9, Z_{11}, Z_{13}, Z_{15}, Z_{17}, and Z_{19} is separately a monosubstituted β-amino acid;

each Z_2, Z_4, Z_6, Z_8, Z_{10}, Z_{12}, Z_{14}, Z_{16}, Z_{18}, and Z_{20} is separately a disubstituted β-amino acid; and

wherein the cyclic β-peptide has a sequence of from three to about ten homochiral β-amino acids, and wherein at least one β-amino acid has at least one polar side chain.

53. The pharmaceceutical composition of any one of claims 1, 29, 30 or 39, wherein the peptide is cyclic [β-Lys- β-Lys- β-Trp- β-Leu- β-Trp- β-Leu] or cyclic [β-Arg- β-Lys- β-Lys- β-Leu- β-Leu -β-Trp].

54. The pharmaceutical composition of any one of claims 1, 29, 30, and 39, wherein said treating is for prevention of said cancer in said animal.

55. The pharmaceutical composition of any one of claims 1, 29, 30, and 39, wherein the cancer is a cancer involving the animal's head, neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin, or central nervous system.

56. The pharmaceutical composition of any one of claims 1, 29, 30, and 39, wherein the target cancer is leukemia, lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, or prostate cancer.

57. The pharmaceutical composition of any one of claims 1, 29, 30, and 39, wherein said treating comprises oral, intraperitoneal or intravenous administration of said pharmaceutical composition to an animal.

58. A method of treating a cancer in an animal comprising administering to said animal a cyclic peptide comprising a sequence of from four to about sixteen amino acids, wherein the sequence has alternating D- and L-α-amino acids, in an amount that does not cause an undesirable amount of animal cell death.

59. The method of claim 58, wherein said treating is for prevention of said cancer in said animal.

60. The method of claim 58, wherein said cancer is a cancer involving the animal's head, neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin, or central nervous system.

61. The method of claim 58, wherein said cancer is leukemia, lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, or prostate cancer.

62. The method of claim 58, wherein said treating comprises oral, intraperitoneal or intravenous administration of said cyclic peptide to an animal.

63. The method of claim 58, wherein the cyclic peptide causes substantially no hemolysis of mammalian red blood cells *in vitro*.

64. The method of claim 58, wherein the cyclic peptide self-assembles into a supramolecular structure comprising a nanotube, a barrel of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof.

65. The method of claim 58, wherein the cyclic peptide has at least one polar D- or L-amino acid.

66. The method of claim 58, wherein the cyclic peptide has at least two polar D- or L-amino acids.

67. The method of claim 58, wherein the cyclic peptide has at least three polar D- or L-amino acids.

68. The method of claim 58, wherein the cyclic peptide has four to six polar D- or L-amino acids.

69. The method of any one of claims 66, 67 and 68, wherein each polar D- or L-amino acid is adjacent to at least one other polar D- or L-amino acid.

70. The method of any one of claims 65, 66, 67 and 68, wherein at least one polar D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids.

71. The method of any one of claims 65, 66, 67 and 68, wherein each polar D- or L-amino acid is separately a D- or L-enantiomer of cysteine, homocysteine, serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, histidine, arginine, lysine, hydroxylysine or ornithine.

72. The method of claim 58, wherein the cyclic peptide has at least one ionizable D- or L-amino acid.

73. The method of claim 58, wherein the cyclic peptide has at least two ionizable D- or L-amino acids.

74. The method of claim 58, wherein the cyclic peptide has at least three ionizable D- or L-amino acids.

75. The method of claim 58, wherein the cyclic peptide has four to six ionizable D- or L-amino acids.

76. The method of any one of claims 73, 74 or 75, wherein each ionizable D- or L-amino acid is adjacent to at least one other polar D- or L-amino acid.

77. The method of any one of claims 72, 73, 74 or 75, wherein at least one ionizable D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids.

78. The method of any one of claims 72, 73, 74 or 75, wherein each ionizable D- or L-amino acid is separately a D- or L-enantiomer of arginine, aspartic acid, glutamic acid, histidine, lysine, hydroxylysine or ornithine.

79. The method of claim 58, wherein the cyclic peptide has at least one nonpolar D- or L-amino acid residue.

80. The method of claim 58, wherein the cyclic peptide has two to fifteen nonpolar D- or L-amino acid residues.

81. The method of claim 79 or 80, wherein each nonpolar D- or L-amino acid residue is a D- or L-alanine, valine, isoleucine, leucine, methionine, norleucine, phenylalanine, tyrosine or tryptophan.

82. A method of treating a cancer in an animal comprising administering to said animal a cyclic peptide comprising a sequence of about six amino acids, wherein the sequence has alternating D- and L-α-amino acids, in an amount that does not cause an undesirable amount of animal cell death.

83. A method of treating a cancer in an animal, comprising administering to said animal a cyclic peptide comprising a sequence of about eight amino acids, wherein the sequence has alternating D- and L-α-amino acids, in an amount that does not cause an undesirable amount of animal cell death.

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84. The method of any one of claims 58, 82 or 83, wherein the cyclic peptide has an amino acid sequence of formula I:

$$\begin{bmatrix} (Y_1)_p\text{-}(X_1)_m\text{-}(Y_2)_p\text{-}(X_2)_p\text{-}(Y_3)_p\text{-}(X_3)_p\text{-}(Y_4)_p\text{-}(X_4)_p\text{-}(Y_5)_p\text{-}(X_5)_p \\ (X_{10})_p\text{-}(Y_{10})_p\text{-}(X_9)_p\text{-}(Y_9)_p\text{-}(X_8)_p\text{-}(Y_8)_p\text{-}(X_7)_p\text{-}(Y_7)_p\text{-}(X_6)_p\text{-}(Y_6)_p \end{bmatrix}$$

I

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid; and

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

85. The method of any one of claims 58, 82 or 83, wherein the cyclic peptide has an amino acid sequence of formula II:

$$\begin{bmatrix} (\text{D-}X_1\text{ -L-}X_2)_m - (\text{D-}Y_1 - \text{L-}Y_2)_p - (\text{L-}X_3 - \text{D-}X_4)_p - (\text{L-}Y_3 - \text{D-}Y_4)_p \\ (\text{L-}Y_8 - \text{D-}Y_7)_p - (\text{D-}X_8\text{ -L-}X_7)_p - (\text{D-}Y_6 - \text{L-}Y_5)_p - (\text{L-}X_6 - \text{D-}X_5)_p \end{bmatrix}$$

II

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, and Y_8 is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

86. The method of any one of claims 58, 82 or 83, wherein the cyclic peptide has an amino acid sequence having formula III:

$$\left[(X_1)_p\text{-}(X_2)_p\text{-}(X_3)_p\text{-}(X_4)_m\text{-}(X_5)_p\text{-}(X_6)_p\text{-}(X_7)_p\text{-}(X_8)_p\text{-}(X_9)_p\text{-}(X_{10})_p\right]$$
$$\left[(Y_{10})_p\text{-}(Y_9)_p\text{-}(Y_8)_p\text{-}(Y_7)_p\text{-}(Y_6)_p\text{-}(Y_5)_p\text{-}(Y_4)_p\text{-}(Y_3)_p\text{-}(Y_2)_p\text{-}(Y_1)_p\right] \qquad \textbf{III}$$

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

87. The method of any one of claims 58, 82 or 83, wherein the cyclic peptide has an amino acid sequence of formula IVa or IVb:

$$\text{D-}X_1 - (\text{L-}X_2 - \text{D-}X_3)_n - (\text{L-}Y_1 - \text{D-}Y_2)_m - \text{L-}Y_3 \qquad \text{IVa}$$

or

$$\text{L-}X_1 - (\text{D-}X_2 - \text{L-}X_3)_n - (\text{D-}Y_1 - \text{L-}Y_2)_m - \text{D-}Y_3 \qquad \text{IVb}$$

wherein:

n is an integer ranging from 0 to 4;

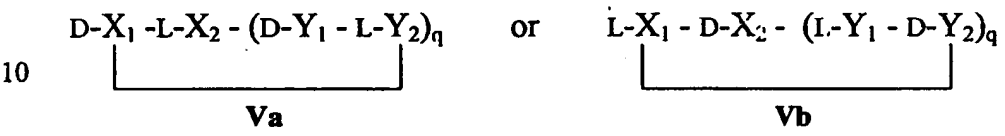
m is an integer ranging from 1 to 7;

X_1, X_2 and X_3 are each a separate a polar amino acid;

Y_1, Y_2 and Y_3 are each a separate nonpolar amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

88. The method of any one of claims 58, 82 or 83, wherein the cyclic peptide has an amino acid sequence of formula Va or Vb:



wherein:

q is an integer ranging from 2 to 7;

X_1 and X_2 are separately polar amino acids;

Y_1 and Y_2 are separately nonpolar amino acids.

89. The method of claim 58, wherein the composition comprises a mixture of two or more different cyclic peptides.

90. The method of claim 58, wherein the composition comprises a mixture of two or more different cyclic peptides.

91. The method of claim 58, wherein the amino acid sequence comprises any one of SEQ ID NO:8, 9, 12, 17, 18, 26, 29, 47-52, 61, 63, 67, 68, 72-77, 84, 85, 87-89, 91-93, 100, 102, 107, 111, 112, 119, 125 or 139.

92. The method of claim 58, wherein the amino acid sequence comprises SEQ ID NO: 72, 85, 91, 93, 125, 126, 133, 145, 146, 147, 148, 149, 151, 152, 155, 156, 157 or a mixture thereof.

93. A method of treating a cancer in an animal comprising administering to said mammal a cyclic peptide comprising a sequence of from three to about ten β-amino acids, in an amount that does not cause an undesirable amount of animal cell death.

94. The method of claim 93, wherein said cancer is a cancer involving the animal's head, neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin, or central nervous system.

95. The method of claim 93, wherein said cancer is leukemia, lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, or prostate cancer.

96. The method of claim 93, wherein said treating comprises oral, intraperitoneal or intravenous administration of said cyclic peptide to an animal.

97. The method of claim 93, wherein the cyclic peptide causes substantially no hemolysis of mammalian red blood cells *in vitro*.

98. The method of claim 93, wherein the amount sufficient to inhibit cancer without inducing an undesired amount of lysis of animal cells.

99. The method of claim 93, wherein the cyclic peptide self-assembles into a supramolecular structure comprising a nanotube, a barrel of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof.

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100. The method of claim 93, wherein the cyclic peptide has at least one polar side chain.

101. The method of claim 93, wherein the cyclic peptide has at least two polar side chains.

102. The method of claim 93, wherein the cyclic peptide has at least three side chains.

103. The method of claim 93, wherein the cyclic peptide has four to five polar side chains.

104. The method of claim 93, wherein the cyclic peptide has at least one ionizable side chain.

105. The method of claim 93, wherein the cyclic peptide has at least two ionizable side chains.

106. The method of claim 93, wherein the cyclic peptide has at least three ionizable side chains.

107. The method of claim 93, wherein the cyclic peptide has four to five ionizable side chains.

108. The method of claim 93, wherein the cyclic peptide has at least one nonpolar side chain.

109. The method of claim 93, wherein the cyclic peptide has two to nine nonpolar side chains.

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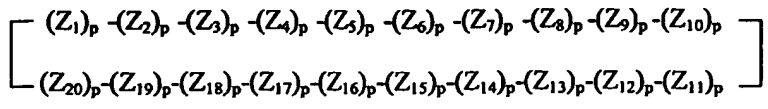
110. The method of claim 93, wherein the cyclic β-peptide can have an amino acid sequence of formula VI:



VI

wherein:

each p is separately an integer ranging from 0 to 7;

each Z_1, Z_3, Z_5, Z_7, Z_9, Z_{11}, Z_{13}, Z_{15}, Z_{17}, and Z_{19} is separately a monosubstituted β-amino acid;

each Z_2, Z_4, Z_6, Z_8, Z_{10}, Z_{12}, Z_{14}, Z_{16}, Z_{18}, and Z_{20} is separately a disubstituted β-amino acid; and

wherein the cyclic β-peptide has a sequence of from three to about ten homochiral β-amino acid side chains.

111. The method of claim 93, wherein the peptide is cyclic [β-Lys- β-Lys- β-Trp- β-Leu- β-Trp- β-Leu] or cyclic [β-Arg- β-Lys- β-Lys- β-Leu- β-Leu -β-Trp].

112. A method for identifying or evaluating a cyclic peptide with anti-cancer activity comprising:

(a) contacting a target cancer with a test cyclic peptide; and

(b) determining whether the test cyclic peptide has anti-cancer activity;

wherein the test cyclic peptide comprises (i) a sequence of from four to about sixteen alternating D- and L- α-amino acids, or (ii) a sequence of from three to about ten β-amino acids.

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113. A method for identifying or evaluating a cyclic peptide selectively cytotoxic to a target cancer comprising:

(a) obtaining a combinatorial library of test cyclic peptides, wherein each test cyclic peptide in the combinatorial library comprises (i) an alternating D- and L- α amino acid sequence of from four to about sixteen amino acids, or (ii) a sequence of from three to about ten β-amino acids;

(b) contacting a target cancer with one or more of the test cyclic peptides; and

(c) determining whether one or more test cyclic peptides have anti-cancer activity.

114. A method of identifying a cyclic peptide selectively cytotoxic to a target cancer, comprising:

(a) identifying a first cyclic peptide from the combinatorial library of the method of claim 113 that can induce cell death of said target cancer;

(a) exchanging at least one amino acid for a different amino acid in the sequence of the first cyclic peptide to generate a second cyclic peptide;

(c) contacting a cancer cell with the second cyclic peptide; and

(d) determining whether the second cyclic peptide has anti-cancer activity.

115. The method of any one of claims 112, 113 or 114, wherein the method further comprises determining whether a cyclic peptide induces an undesired amount of lysis of an animal cell type.

116. The method of claim 115, wherein the animal cell type is a mammalian red blood cell.

117. The method of any one of claims 112, 113 or 114, wherein the method further comprises determining whether a cyclic peptide induces substantial cell death in another animal cell type.

118. The method of any one of claims 112, 113 or 114, wherein the contacting step is performed *in vitro*.

119. The method of any one of claims 112, 113 or 114, wherein the contacting step is performed *in vivo*.

120. A peptide comprising a cyclic amino acid sequence of from four to about sixteen alternating D- and L-α-amino acids, said peptide having anti-cancer activity but substantially no undesired anti-animal cell activity.

121. The peptide of claim 120, wherein the peptide has a minimum inhibitory concentration at which substantially no target cancers grow *in vitro* that is less than about one twentieth to about one half of the peptide concentration needed to cause 50% hemolysis of mammalian red blood cells.

122. The peptide of claim 120, wherein the peptide causes substantially no hemolysis of mammalian red blood cells *in vitro*.

123. The peptide of claim 120, wherein the peptide has anti-cancer activity against a cancer involving the animal's head, neck, lung, mesothelioma, mediastinum, esophagus, stomach, pancreas, hepatobiliary system, small intestine, colon, colorectal, rectum, anus, kidney, urethra, bladder, prostate, urethra, penis, testis, gynecological organs, ovaries, breast, endocrine system, skin, or central nervous system.

124. The peptide of claim 120, wherein the peptide has anti-cancer activity against leukemia, lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, or prostate cancer.

125. The peptide of claim 120, wherein the cyclic peptide self-assembles into a supramolecular structure more disruptive of target viral membranes than of animal cell membranes.

126. The peptide of claim 120, wherein the supramolecular structure comprises a nanotube, a barrel of associated, axially parallel nanotubes, a carpet of associated nanotubes, or mixtures thereof.

127. The peptide of claim 120, wherein the supramolecular structure induces cancer cell death or growth inhibition selectively over animal cell lysis.

128. The peptide of claim 120, wherein the peptide has at least one polar D- or L-amino acid.

129. The peptide of claim 120, wherein the peptide has at least two polar D- or L-amino acids.

130. The peptide of claim 120, wherein the peptide has at least three polar D- or L-amino acids.

131. The peptide of claim 120, wherein the peptide has four to six polar D- or L-amino acids.

132. The peptide of any one of claims 129, 130 or 131, wherein each polar D- or L-amino acid is adjacent to at least one other polar D- or L-amino acid.

133. The peptide of any one of claims 128, 129, 130 or 131, wherein at least one polar D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids.

134. The peptide of any one of claims 128, 129, 130 or 131, wherein each polar D- or L-amino acid is separately a D- or L-enantiomer of cysteine, homocysteine, serine, threonine, asparagine, glutamine, aspartic acid, glutamic acid, histidine, arginine, lysine, hydroxylysine or ornithine.

135. The peptide of claim 120, wherein the cyclic peptide has at least one ionizable D- or L-amino acid.

136. The peptide of claim 120, wherein the cyclic peptide has at least two ionizable D- or L-amino acids.

137. The peptide of claim 120, wherein the cyclic peptide has at least three ionizable D- or L-amino acids.

138. The peptide of claim 120, wherein the cyclic peptide has four to six ionizable D- or L-amino acids.

139. The peptide of any one of claims 136, 137 or 138, wherein each ionizable D- or L-amino acid is adjacent to at least one polar D- or L-amino acid.

140. The peptide of any one of claims 135, 136, 137 or 138, wherein at least one ionizable D- or L-amino acid is adjacent only to nonpolar D- or L-amino acids

141. The peptide of any one of claims 135, 136, 137 or 138, wherein each ionizable D- or L-amino acid is separately arginine, aspartic acid, glutamic acid, histidine, lysine, hydroxylysine or ornithine.

142. The peptide of claim 120, wherein the cyclic peptide has at least one nonpolar D- or L-amino acid residue.

143. The peptide of claim 120, wherein the cyclic peptide has two to fifteen nonpolar D- or L-amino acid residues.

144. The peptide of claim 142 or 143, wherein each nonpolar D- or L-amino acid residue is alanine, valine, isoleucine, leucine, methionine, norleucine, phenylalanine, tyrosine or tryptophan.

145. A peptide comprising a cyclic amino acid sequence of about six alternating D- and L-α-amino acids, said peptide having anti-cancer activity but substantially no anti-animal cell activity.

146. A peptide comprising a cyclic amino acid sequence of about eight alternating D- and L-α-amino acids, said peptide having anti-cancer activity but substantially no anti-animal cell activity.

147. The peptide of any one of claims 120, 145 or 146, wherein the cyclic peptide has an amino acid sequence of formula I:

$$\left[(Y_1)_p\text{-}(X_1)_m\text{-}(Y_2)_p\text{-}(X_2)_p\text{-}(Y_3)_p\text{-}(X_3)_p\text{-}(Y_4)_p\text{-}(X_4)_p\text{-}(Y_5)_p\text{-}(X_5)_p\text{-}(X_{10})_p\text{-}(Y_{10})_p\text{-}(X_9)_p\text{-}(Y_9)_p\text{-}(X_8)_p\text{-}(Y_8)_p\text{-}(X_7)_p\text{-}(Y_7)_p\text{-}(X_6)_p\text{-}(Y_6)_p\right]$$

I

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid; and

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid;

wherein the cyclic peptide has a sequence comprising an even number of from four to about sixteen alternating D- and L-α amino acids.

148. The peptide of any one of claims 120, 145 or 146, wherein the cyclic peptide has an amino acid sequence of formula II:

$$\begin{bmatrix} (D\text{-}X_1\text{-}L\text{-}X_2)_m - (D\text{-}Y_1 - L\text{-}Y_2)_p - (L\text{-}X_3 - D\text{-}X_4)_p - (L\text{-}Y_3 - D\text{-}Y_4)_p \\ (L\text{-}Y_8 - D\text{-}Y_7)_p - (D\text{-}X_8\text{-}L\text{-}X_7)_p - (D\text{-}Y_6\text{-}L\text{-}Y_5)_p - (L\text{-}X_6 - D\text{-}X_5)_p \end{bmatrix}$$

II

wherein:

m is an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, and X_8 is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, and Y_8 is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

149. The peptide of any one of claims 120, 145 or 146, wherein the peptide has an amino acid sequence having formula III:

$$\begin{bmatrix} (X_1)_p\text{-}(X_2)_p\text{-}(X_3)_p\text{-}(X_4)_m\text{-}(X_5)_p\text{-}(X_6)_p\text{-}(X_7)_p\text{-}(X_8)_p\text{-}(X_9)_p\text{-}(X_{10})_p \\ (Y_{10})_p\text{-}(Y_9)_p\text{-}(Y_8)_p\text{-}(Y_7)_p\text{-}(Y_6)_p\text{-}(Y_5)_p\text{-}(Y_4)_p\text{-}(Y_3)_p\text{-}(Y_2)_p\text{-}(Y_1)_p \end{bmatrix}$$

III

wherein:

each m is separately an integer ranging from 1 to 7;

each p is separately an integer ranging from 0 to 7;

each X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, and X_{10} is separately a polar D- or L-α-amino acid;

each Y_1, Y_2, Y_3, Y_4, Y_5, Y_6, Y_7, Y_8, Y_9, and Y_{10} is separately nonpolar D- or L-α-amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

150. The peptide of any one of claims 120, 145 or 146, wherein the peptide has an amino acid sequence of formula IVa or IVb:

$$D\text{-}X_1 - (L\text{-}X_2 - D\text{-}X_3)_n - (L\text{-}Y_1 - D\text{-}Y_2)_m - L\text{-}Y_3 \qquad \text{IVa}$$

or

$$L\text{-}X_1 - (D\text{-}X_2 - L\text{-}X_3)_n - (D\text{-}Y_1 - L\text{-}Y_2)_m - D\text{-}Y_3 \qquad \text{IVb}$$

wherein:

n is an integer ranging from 0 to 4;

m is an integer ranging from 1 to 7;

X_1, X_2 and X_3 are each a separate a polar amino acid;

Y_1, Y_2 and Y_3 are each a separate nonpolar amino acid; and

wherein the cyclic peptide has an even number of from four to about sixteen alternating D- and L-α amino acids.

151. The peptide of any one of claims 120, 145 or 146, wherein the peptide has an amino acid sequence of formula Va or Vb:

$$D\text{-}X_1\text{-}L\text{-}X_2 - (D\text{-}Y_1 - L\text{-}Y_2)_q \qquad \text{Va}$$

or

$$L\text{-}X_1 - D\text{-}X_2 - (L\text{-}Y_1 - D\text{-}Y_2)_q \qquad \text{Vb}$$

wherein:

q is an integer ranging from 2 to 7;

X_1 and X_2 are separately polar amino acids;

Y_1 and Y_2 are separately nonpolar amino acids.

152. The peptide of claim 120, wherein the amino acid sequence comprises any one of SEQ ID NO:8, 9, 12, 17, 18, 26, 29, 47-52, 61, 63, 67, 68, 72-77, 84, 85, 87-89, 91-93, 100, 102, 107, 111, 112, 119, 125 or 139.

153. The peptide of claim 120, wherein the amino acid sequence comprises any one of SEQ ID NO:72, 85, 91, 93, 125, 126, 135, 145, 146, 147, 148, 149, 151, 152, 155, 156, 157 or a mixture thereof.

154. The pharmaceutical composition of any of claims 1, 29, 30 or 39, wherein the animal is a human.

155. The pharmaceutical composition of any of claims 1, 29, 30 or 39, wherein the animal is a farm animal.

156. The pharmaceutical composition of any of claims 1, 29, 30 or 39, wherein the animal is a domestic animal.

157. The method of claim 58, wherein the animal is a human.

158. The method of claim 58, wherein the animal is a farm animal.

159. The method of claim 58, wherein the animal is a domestic animal.

160. The peptide of any of claims 120, 145 or 146, that is substantially non-toxic in mammals.

161. The peptide of any of claim 120, 145 or 146, that is substantially non-toxic in humans.

162. The peptide of any of claims 120, 145 or 146, that is substantially non-toxic in a target animal in which the peptide is intended for use to treat or prevent a cancer.

163. A method of identifying cyclic peptides useful for treating a cancer comprising:

(a) contacting a target cancer with one or more test cyclic peptides; and

(b) determining whether one or more of the test cyclic peptides have anti-cancer activity; wherein the test cyclic peptides comprises one more of (i) a sequence of from four to about sixteen alternating D- and L- a-amino acids, or (ii) a sequence of from three to about ten β-amino acids.

164. A method of identifying a cyclic peptide selectively cytotoxic to a target cancer cell-type comprising:

(a) contacting said target cancer cell-type with a test cyclic peptide comprising a sequence of from four to about sixteen alternating D- and L- α amino acids, or of from three to about ten β-amino acids; and

(b) determining whether said test cyclic peptide induces cell death of said target cancer cell-type without inducing substantial or undesired cell death in a second cell type.

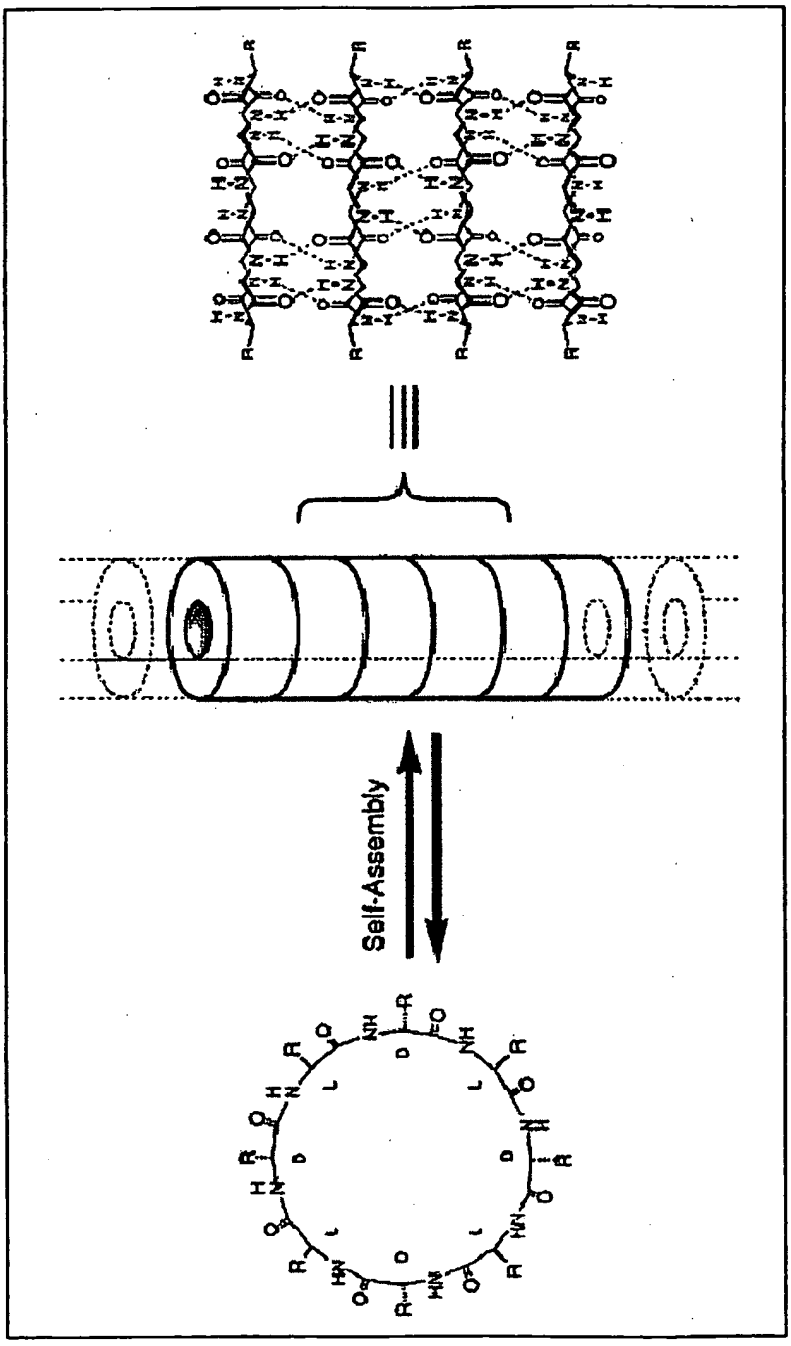

Fig. 1

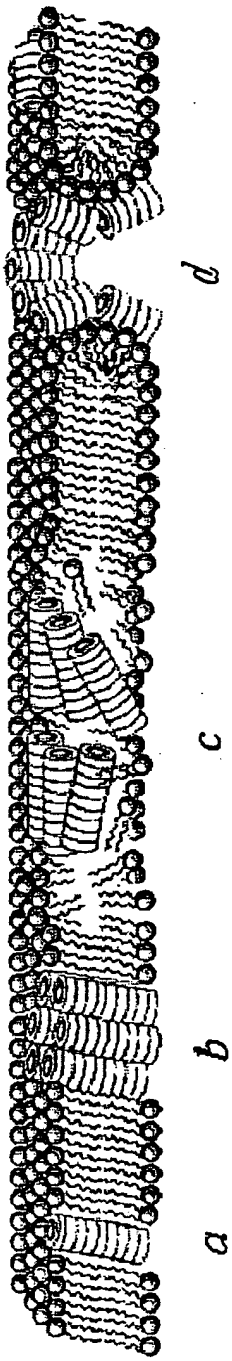

Fig.2

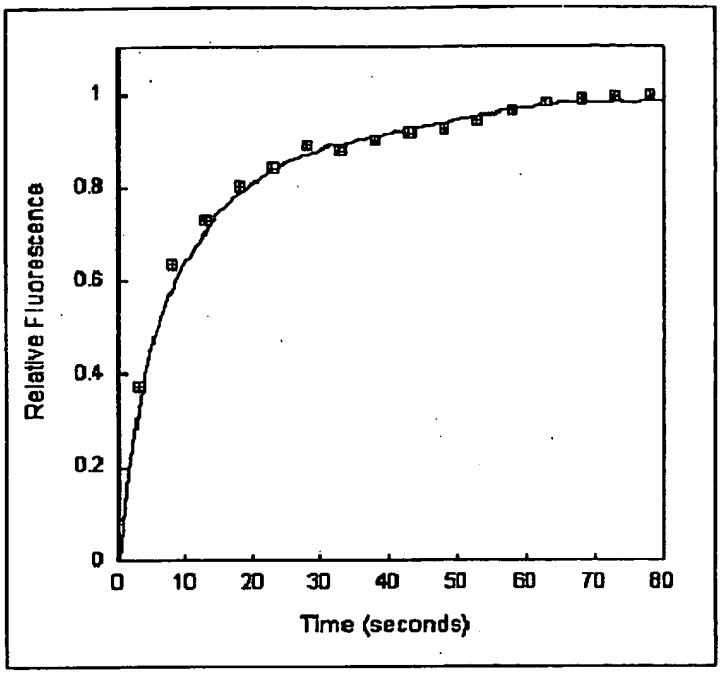
Relative Fluorescence
0
0.2
0.4
0.6
0.8
1
0
10
20
30
40
50
60
70
80
Time (seconds)

Fig. 3A

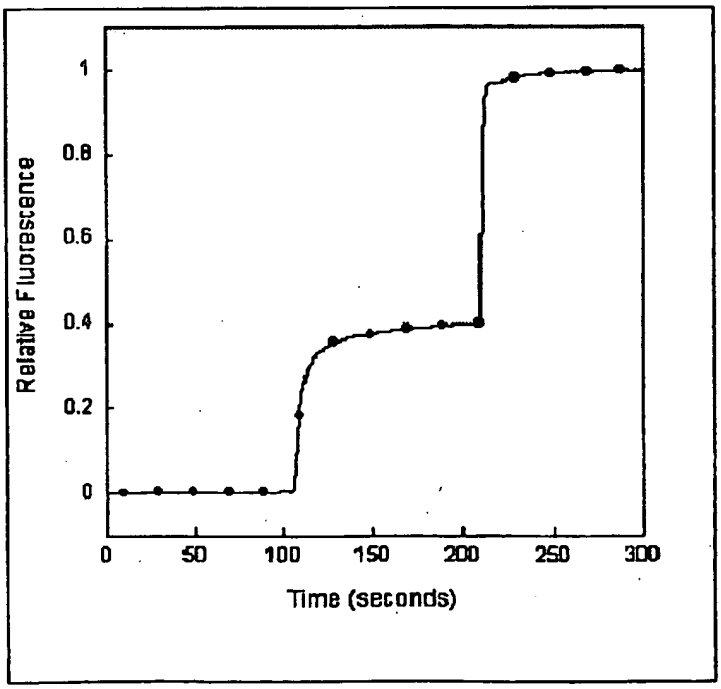
Relative Fluorescence
0
0.2
0.4
0.6
0.8
1
0
50
100
150
200
250
300
Time (seconds)

Fig. 3B

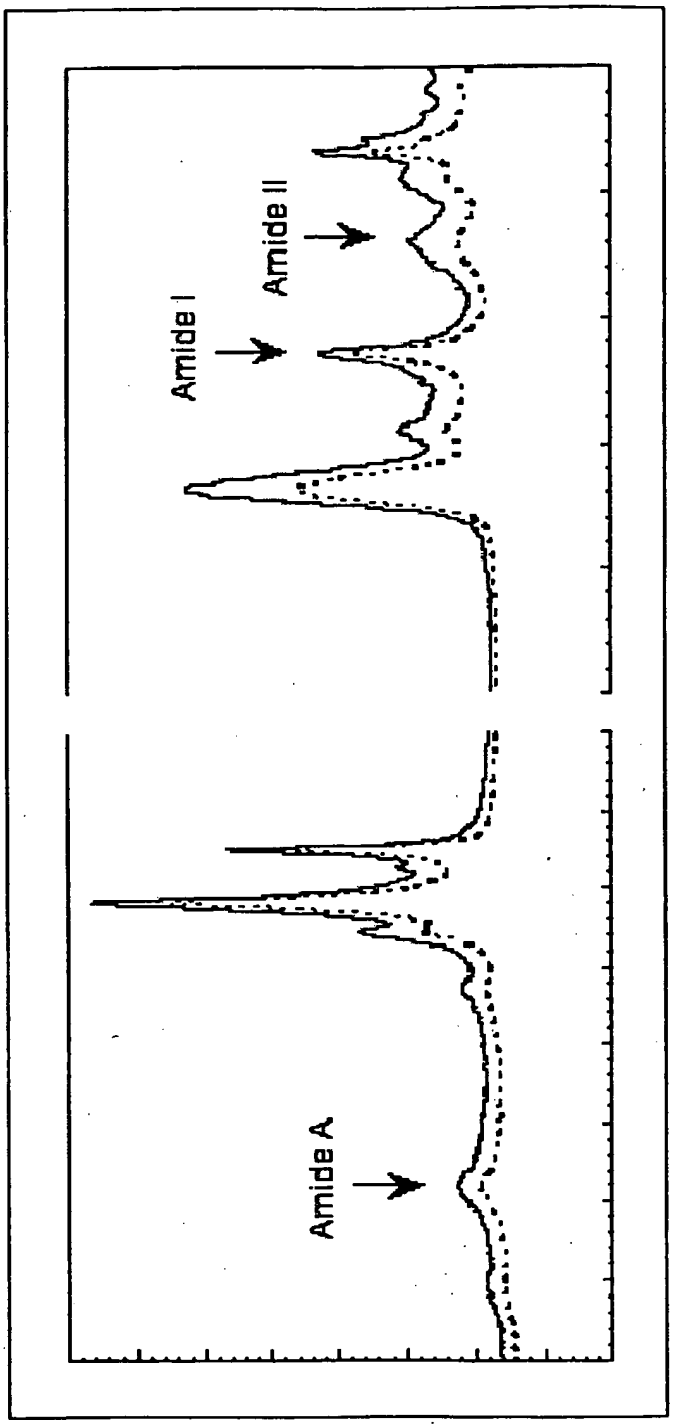

Fig. 4

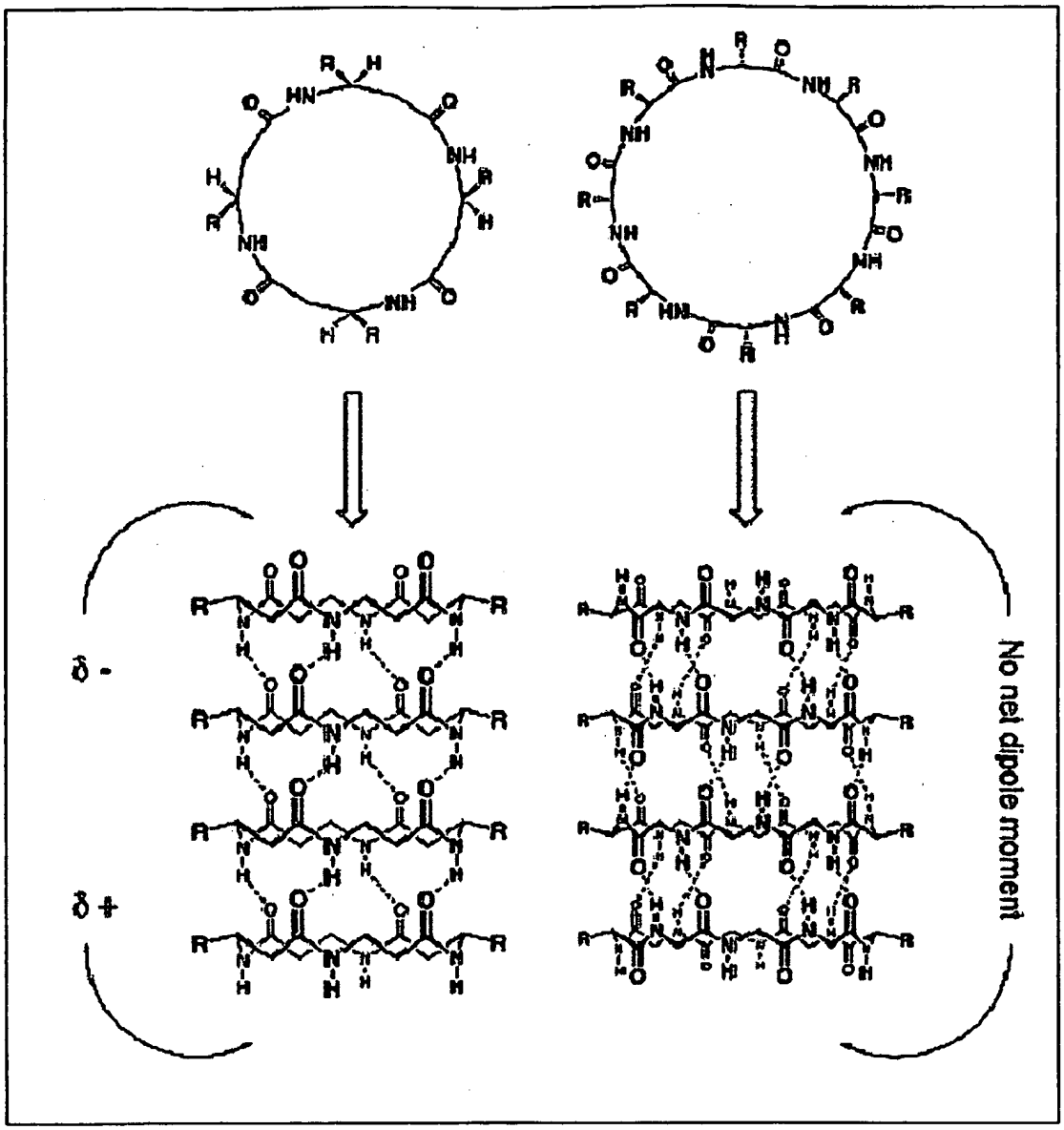

Fig. 5A Fig. 5B

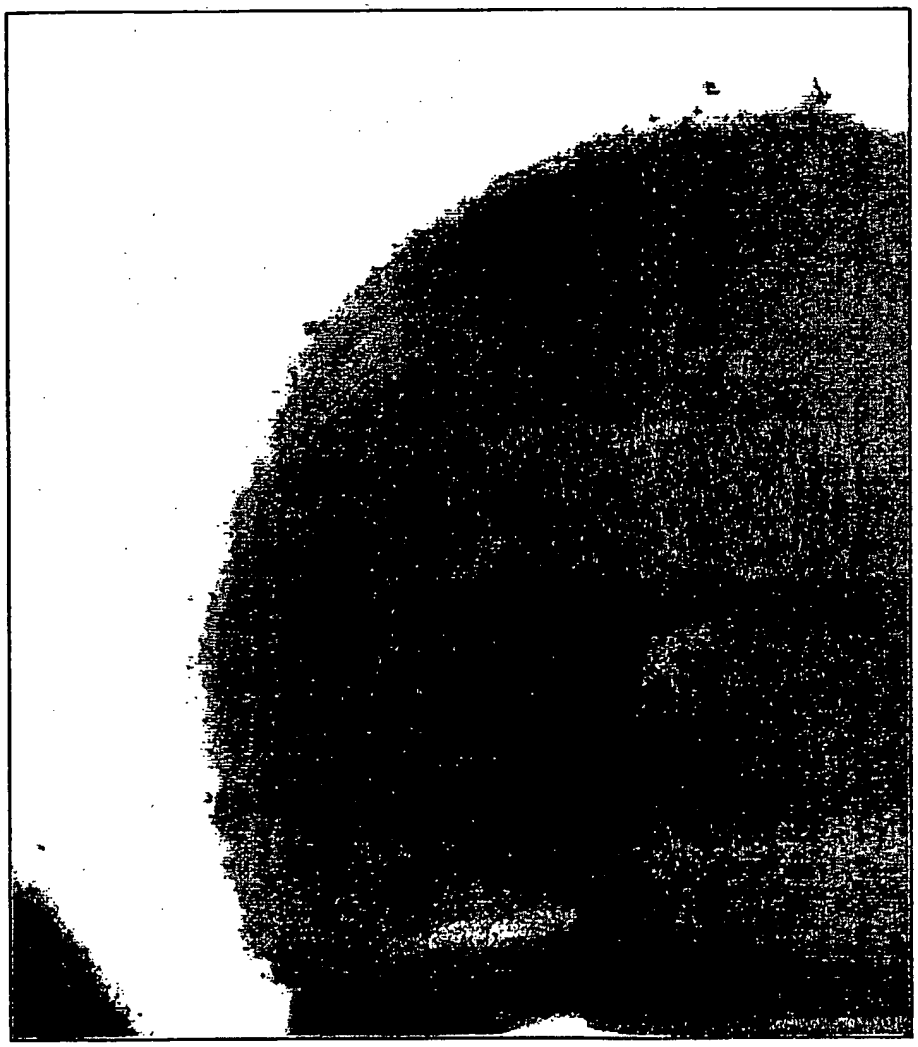

Fig. 6

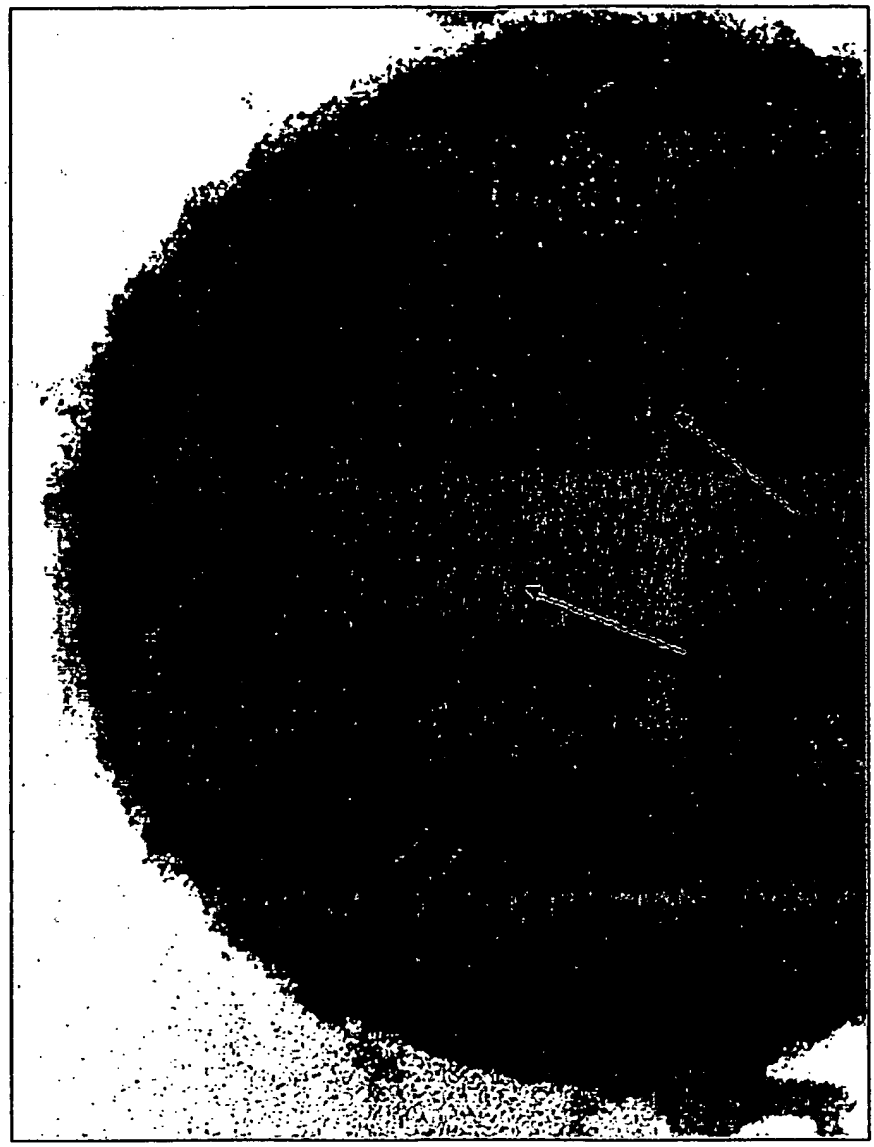

Fig. 7

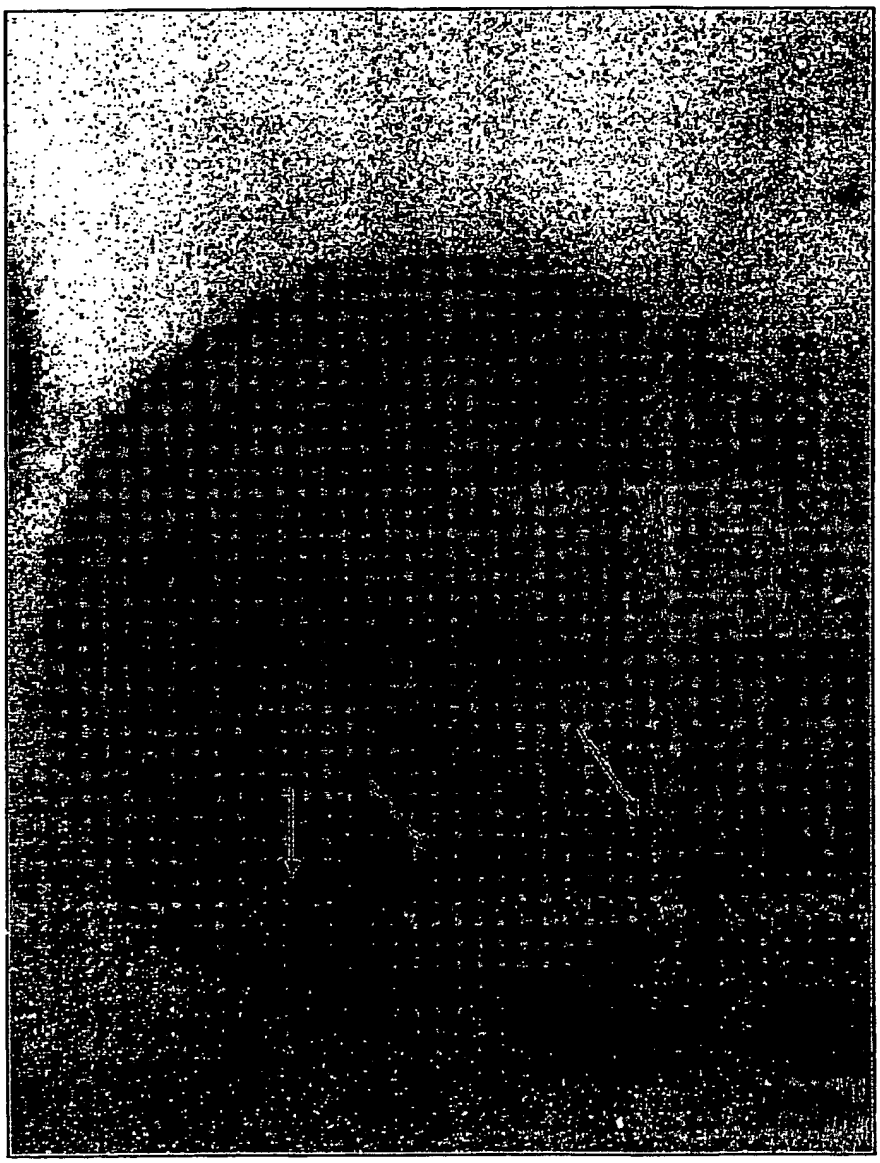

Fig. 8

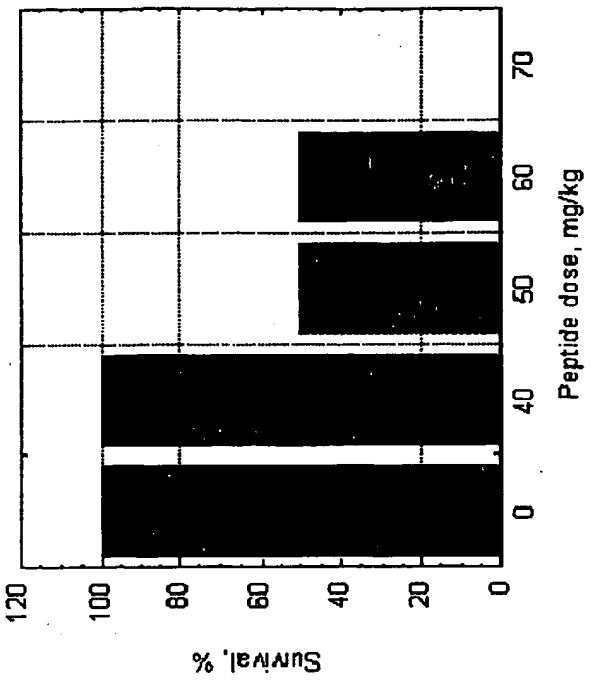
Survival, %
120
100
80
60
40
20
0
Peptide dose, mg/kg
0
40
50
60
70

Fig. 9B

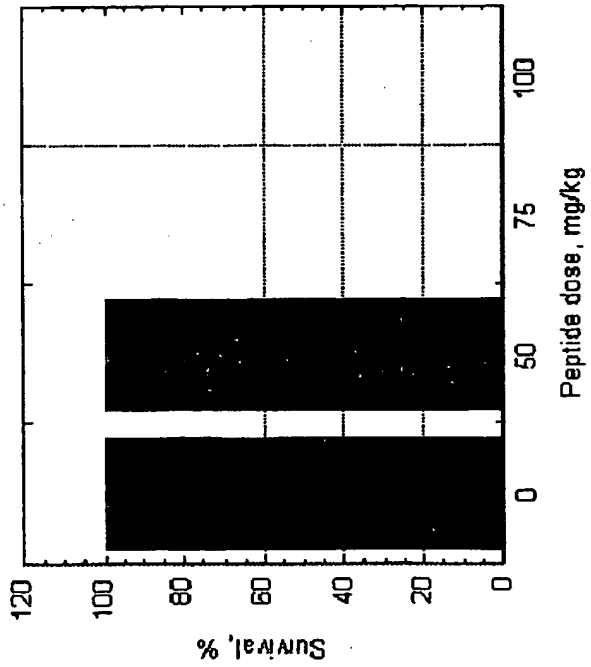
Survival, %
120
100
80
60
40
20
0
Peptide dose, mg/kg
0
50
75
100

Fig. 9A

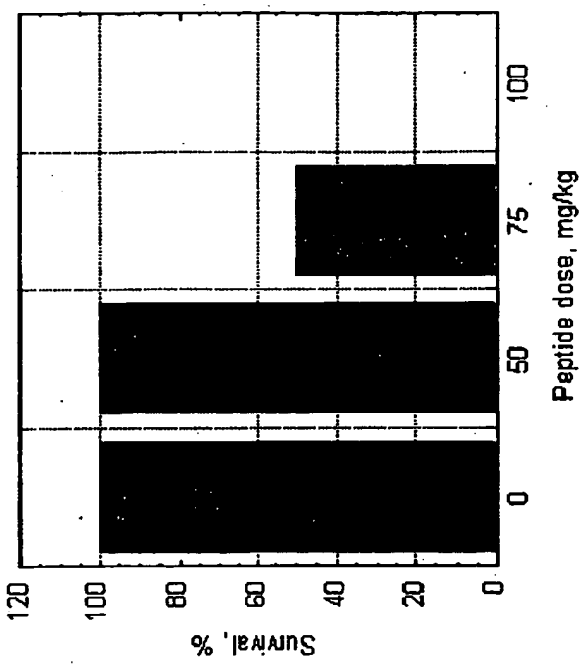

Fig. 10B

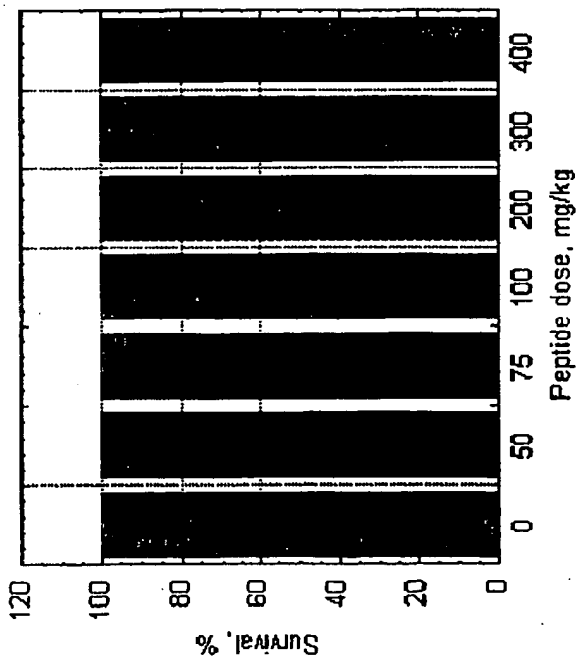

Fig. 10A

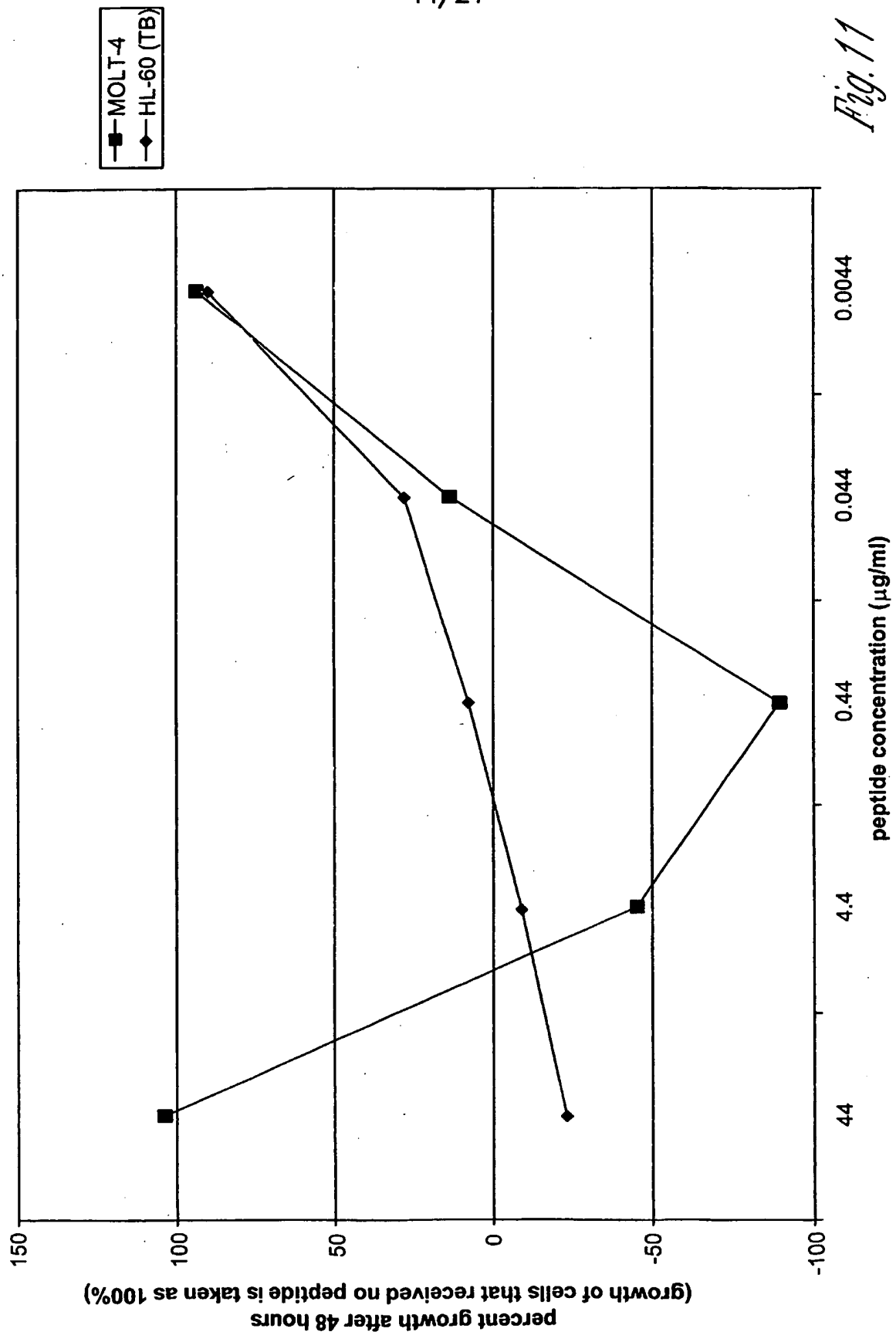
MOLT-4
HL-60 (TB)
150
100
50
0
-50
-100
percent growth after 48 hours
(growth of cells that received no peptide is taken as 100%)
44
4.4
0.44
0.044
0.0044
peptide concentration (µg/ml)

Fig. 11

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC: D- 723334 / 1	Experiment ID: 0203NS65-5	Test Type: 08	Units: Molar
Report Date: April 11, 2002	Test Date: March 11, 2002	QNS:	MC:
COMI: SH-KSK (12906)	Stain Reagent: SRB Dual-Pass	SSPL: 0LVI	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.3	-7.3	-6.3	-5.3	-4.3	-8.3	-7.3	-6.3	-5.3	-4.3	GI50	TGI	LC50
Leukemia															
HL-60(TB)	0.850	2.597	2.420	1.334	0.998	0.771	0.658	90	28	8	-9	-23	2.19E-08	1.52E-06	>5.00E-05
K-562	0.269	1.245	1.121	0.940	0.756	1.014	0.892	87	69	50	76	64	.	>5.00E-05	>5.00E-05
MOLT-4	0.239	0.618	0.473	0.374	0.122	0.115	0.270	62	36	-45	-52	8	1.41E-08	.	.
SR	0.289	0.477	0.297	0.250	0.008	0.186	0.528	4	-14	-97	-36	127	.	.	.
Non-Small Cell Lung Cancer															
A549/ATCC	0.289	0.952	0.774	0.703	0.652	0.770	0.697	73	62	55	73	62	>5.00E-05	>5.00E-05	>5.00E-05
EKVX	0.398	0.674	0.571	0.534	0.461	0.600	0.718	63	49	23	73	116	.	>5.00E-05	>5.00E-05
HOP-62	0.482	0.874	0.688	0.748	0.754	0.785	1.125	52	68	69	77	164	>5.00E-05	>5.00E-05	>5.00E-05
HOP-92	1.129	1.443	1.244	1.329	1.237	1.333	1.725	37	64	34	65	190	.	>5.00E-05	>5.00E-05
NCI-H226	0.288	0.474	0.358	0.380	0.425	0.463	0.540	38	49	73	94	135	.	>5.00E-05	>5.00E-05
NCI-H23	0.750	1.223	1.087	1.114	1.093	1.121	1.298	71	77	73	79	116	>5.00E-05	>5.00E-05	>5.00E-05
NCI-H322M	0.328	1.306	1.178	1.166	1.154	1.211	1.241	87	86	84	90	93	>5.00E-05	>5.00E-05	>5.00E-05
NCI-H460	1.138	2.400	2.227	2.356	2.295	2.388	2.056	86	96	92	99	73	>5.00E-05	>5.00E-05	>5.00E-05
NCI-H522															
Colon Cancer															
COLO 205	0.219	1.371	1.252	1.148	1.200	1.344	0.958	90	81	85	98	64	>5.00E-05	>5.00E-05	>5.00E-05
HCT-116	0.158	0.670	0.434	0.550	0.563	0.591	0.610	54	77	79	85	88	>5.00E-05	>5.00E-05	>5.00E-05
HCT-15	0.422	1.328	1.197	1.128	1.115	1.249	1.234	85	78	76	91	90	>5.00E-05	>5.00E-05	>5.00E-05
HT29	0.188	1.052	1.004	0.930	0.807	0.956	0.544	94	86	72	89	41	3.26E-05	>5.00E-05	>5.00E-05
KM12	0.427	1.250	1.123	1.214	1.186	0.928	0.891	85	96	92	61	56	>5.00E-05	>5.00E-05	>5.00E-05
SW-620	0.295	0.963	0.870	0.827	0.732	0.835	0.968	86	80	65	81	101	>5.00E-05	>5.00E-05	>5.00E-05
CNS Cancer															
SF-268	0.589	1.466	1.240	1.293	1.236	1.269	1.520	74	80	74	78	106	>5.00E-05	>5.00E-05	>5.00E-05
SF-295	0.547	1.341	1.207	1.301	1.219	1.477	1.526	83	95	85	117	123	>5.00E-05	>5.00E-05	>5.00E-05
SF-539	0.408	1.684	1.580	1.555	1.472	1.540	0.875	92	90	83	89	37	2.76E-05	>5.00E-05	>5.00E-05
SNB-19	0.441	1.182	1.052	1.022	0.925	1.078	1.077	83	78	65	86	86	>5.00E-05	>5.00E-05	>5.00E-05
SNB-75	0.377	0.996	0.885	0.891	0.852	0.930	1.297	82	83	77	89	149	>5.00E-05	>5.00E-05	>5.00E-05
U251	0.296	1.458	1.379	1.307	1.229	1.143	1.084	93	87	80	73	68	>5.00E-05	>5.00E-05	>5.00E-05
Melanoma															
LOX IMVI	0.300	1.442	1.359	1.281	1.303	1.397	1.507	93	86	88	87	106	>5.00E-05	>5.00E-05	>5.00E-05
MALME-3M	0.596	0.889	0.738	0.780	0.773	0.835	0.736	48	63	60	81	48	.	>5.00E-05	>5.00E-05
M14	0.345	0.929	0.776	0.884	0.844	0.985	1.147	74	92	85	110	137	>5.00E-05	>5.00E-05	>5.00E-05
SK-MEL-2	0.617	1.274	1.257	1.323	1.297	1.267	1.567	97	107	104	99	145	>5.00E-05	>5.00E-05	>5.00E-05
SK-MEL-28	0.459	1.659	1.635	1.611	1.441	1.759	1.234	98	96	82	108	65	>5.00E-05	>5.00E-05	>5.00E-05
SK-MEL-5	0.469	0.936	0.781	0.827	0.658	0.916	0.767	67	77	40	96	64	.	>5.00E-05	>5.00E-05
UACC-257	1.078	1.368	1.219	1.131	1.154	1.324	1.164	48	18	26	85	30	.	>5.00E-05	>5.00E-05
UACC-62	0.690	1.609	1.362	1.734	1.692	2.057	1.234	73	114	109	169	59	>5.00E-05	>5.00E-05	>5.00E-05
Ovarian Cancer															
IGROV1	0.381	1.258	1.104	1.188	1.104	1.190	1.228	82	92	82	92	97	>5.00E-05	>5.00E-05	>5.00E-05
OVCAR-3	0.413	0.925	0.779	0.854	0.848	0.897	1.154	71	86	85	95	145	>5.00E-05	>5.00E-05	>5.00E-05
OVCAR-4	0.216	0.759	0.625	0.604	0.547	0.663	0.681	75	71	61	82	86	>5.00E-05	>5.00E-05	>5.00E-05
OVCAR-5	0.436	0.806	0.713	0.700	0.744	0.832	0.918	75	71	83	107	130	>5.00E-05	>5.00E-05	>5.00E-05
OVCAR-8	0.586	1.209	1.127	1.016	1.032	1.092	1.015	87	69	72	81	69	>5.00E-05	>5.00E-05	>5.00E-05
SK-OV-3	0.255	1.155	1.051	1.033	0.939	1.082	1.476	88	86	76	92	136	>5.00E-05	>5.00E-05	>5.00E-05
Renal Cancer															
786-0	0.324	1.001	0.850	0.826	0.857	0.940	1.058	78	74	79	91	108	>5.00E-05	>5.00E-05	>5.00E-05
ACHN	0.318	1.157	1.054	1.053	1.021	1.161	1.362	88	88	84	101	125	>5.00E-05	>5.00E-05	>5.00E-05
CAKI-1	0.459	0.668	0.465	0.497	0.490	0.662	1.156	3	18	15	97	334	.	>5.00E-05	>5.00E-05
RXF 393	0.446	0.870	0.673	0.721	0.752	0.740	1.042	53	65	72	69	141	>5.00E-05	>5.00E-05	>5.00E-05
SN12C	0.564	1.579	1.501	1.467	1.509	1.508	1.757	92	89	93	93	117	>5.00E-05	>5.00E-05	>5.00E-05
TK-10	0.551	1.043	0.944	0.960	0.941	1.040	0.793	80	83	79	99	49	4.79E-05	>5.00E-05	>5.00E-05
UO-31	0.560	1.616	1.481	1.528	1.484	1.434	1.622	87	92	88	83	101	>5.00E-05	>5.00E-05	>5.00E-05
Prostate Cancer															
PC-3	0.504	1.596	1.493	1.455	1.322	1.558	1.515	91	87	75	97	93	>5.00E-05	>5.00E-05	>5.00E-05
DU-145	0.282	0.643	0.625	0.525	0.493	0.638	0.665	95	67	58	99	106	>5.00E-05	>5.00E-05	>5.00E-05
Breast Cancer															
MCF7	0.610	1.387	1.253	1.196	1.240	1.262	1.387	83	75	81	84	100	>5.00E-05	>5.00E-05	>5.00E-05
NCI/ADR-RES	0.547	1.180	1.085	1.111	1.084	1.098	1.310	85	89	85	87	121	>5.00E-05	>5.00E-05	>5.00E-05
MDA-MB-231/ATCC	0.564	1.197	1.138	1.066	1.059	1.201	0.452	91	79	78	101	-20	1.32E-05	3.42E-05	>5.00E-05
HS 578T	0.813	1.288	1.170	1.172	1.139	1.339	1.565	75	76	69	111	158	>5.00E-05	>5.00E-05	>5.00E-05
MDA-MB-435	0.504	1.320	1.198	1.255	1.341	1.342	1.482	85	92	103	103	120	>5.00E-05	>5.00E-05	>5.00E-05
T-47D	0.552	1.031	0.959	0.865	0.811	1.053	0.865	85	65	54	104	65	>5.00E-05	>5.00E-05	>5.00E-05

Fig. 12

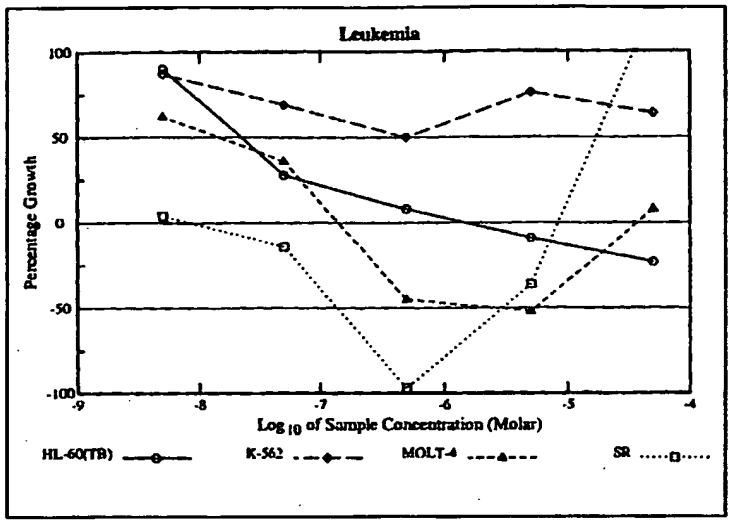

Fig. 13A

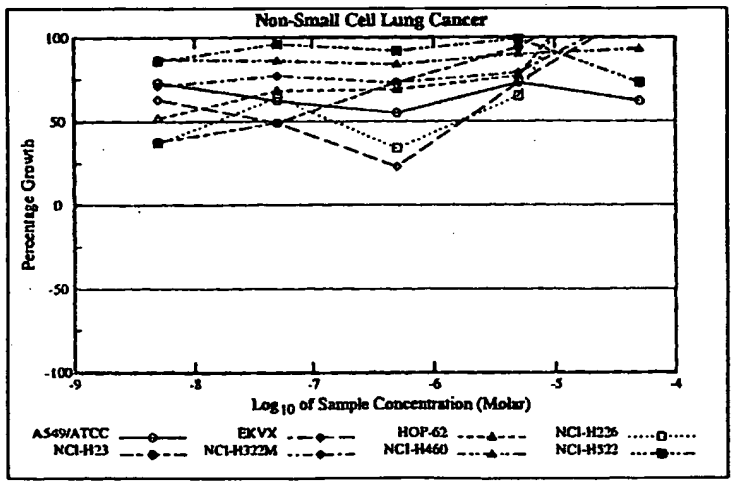

Fig. 13B

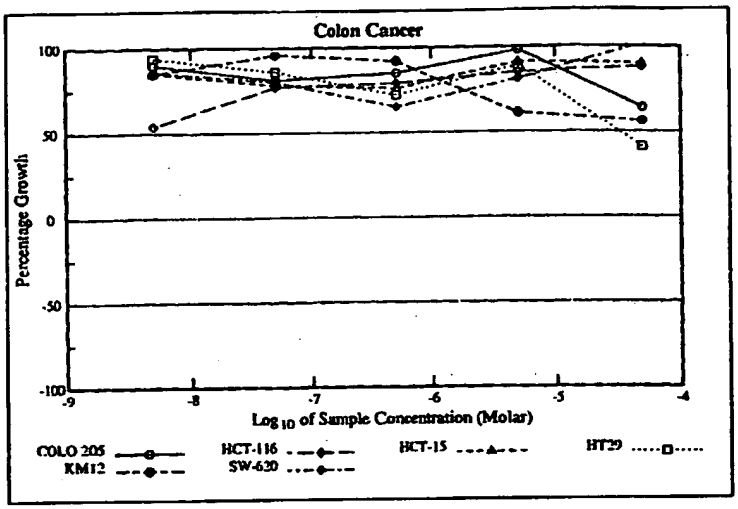

Fig. 13C

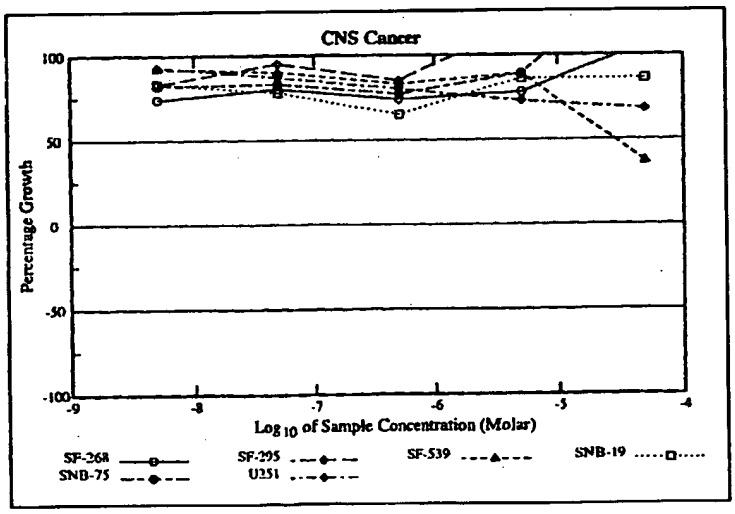

Fig. 13D

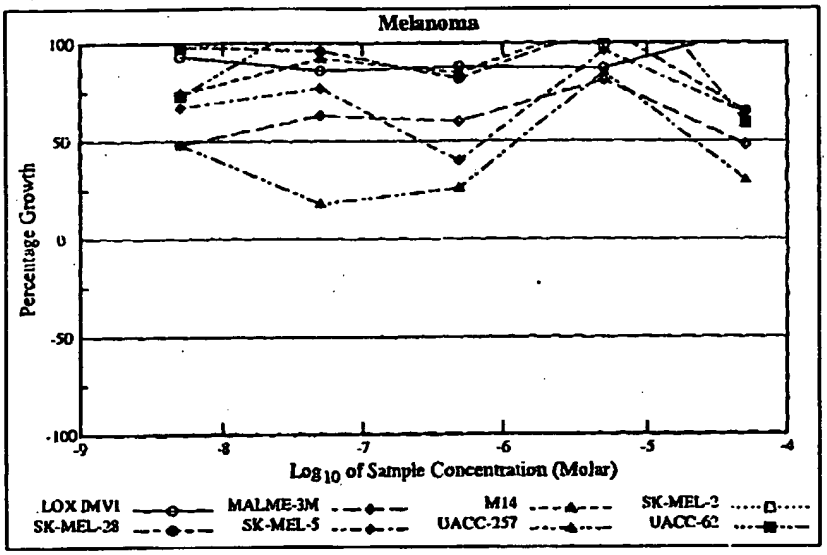

Fig. 13E

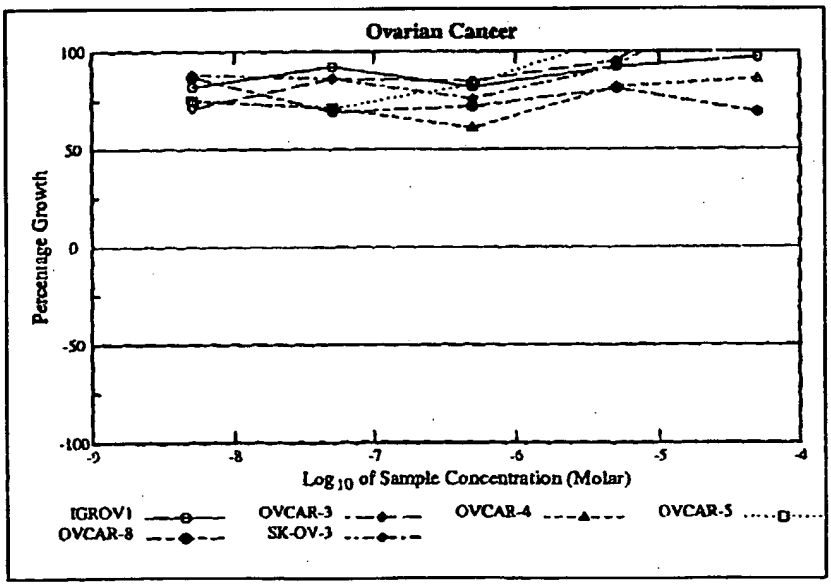

Fig. 13F

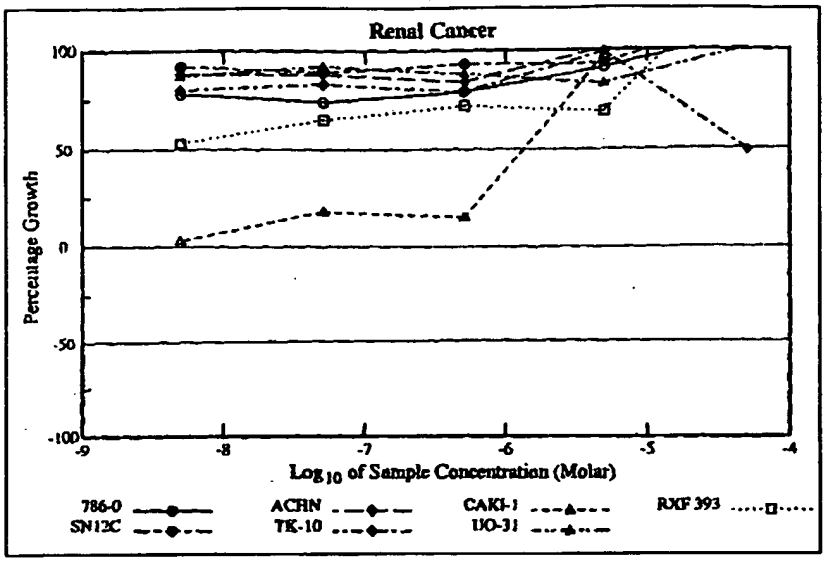

Fig. 13G

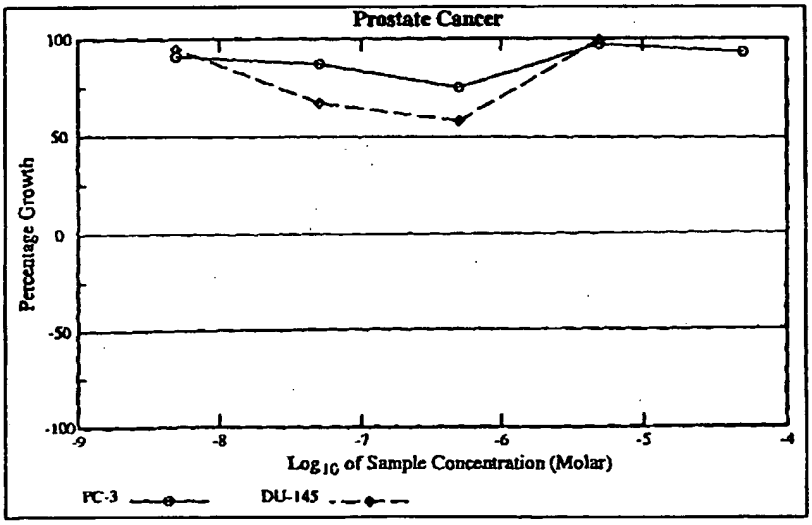

Fig. 13H

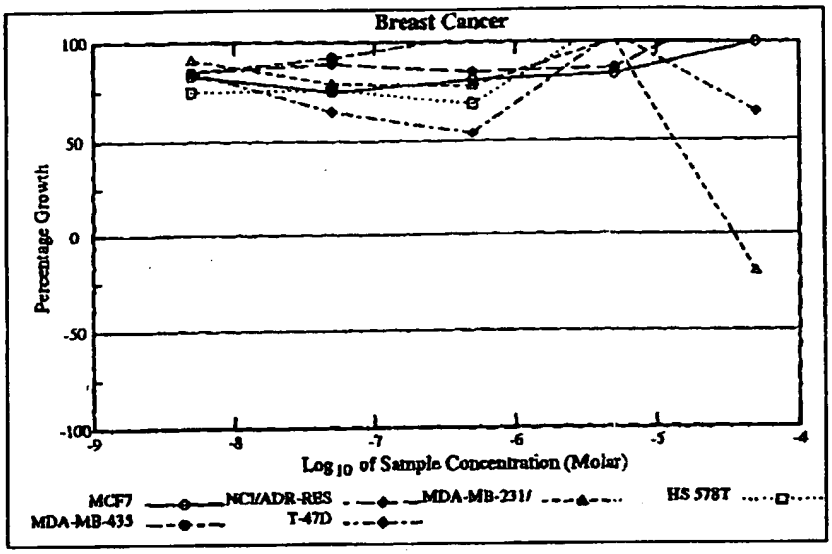
Breast Cancer
100
50
0
-50
-100
Percentage Growth
-9
-8
-7
-6
-5
-4
Log 10 of Sample Concentration (Molar)
MCF7
NCI/ADR-RES
MDA-MB-231/
HS 578T
MDA-MB-435
T-47D

Fig. 13I

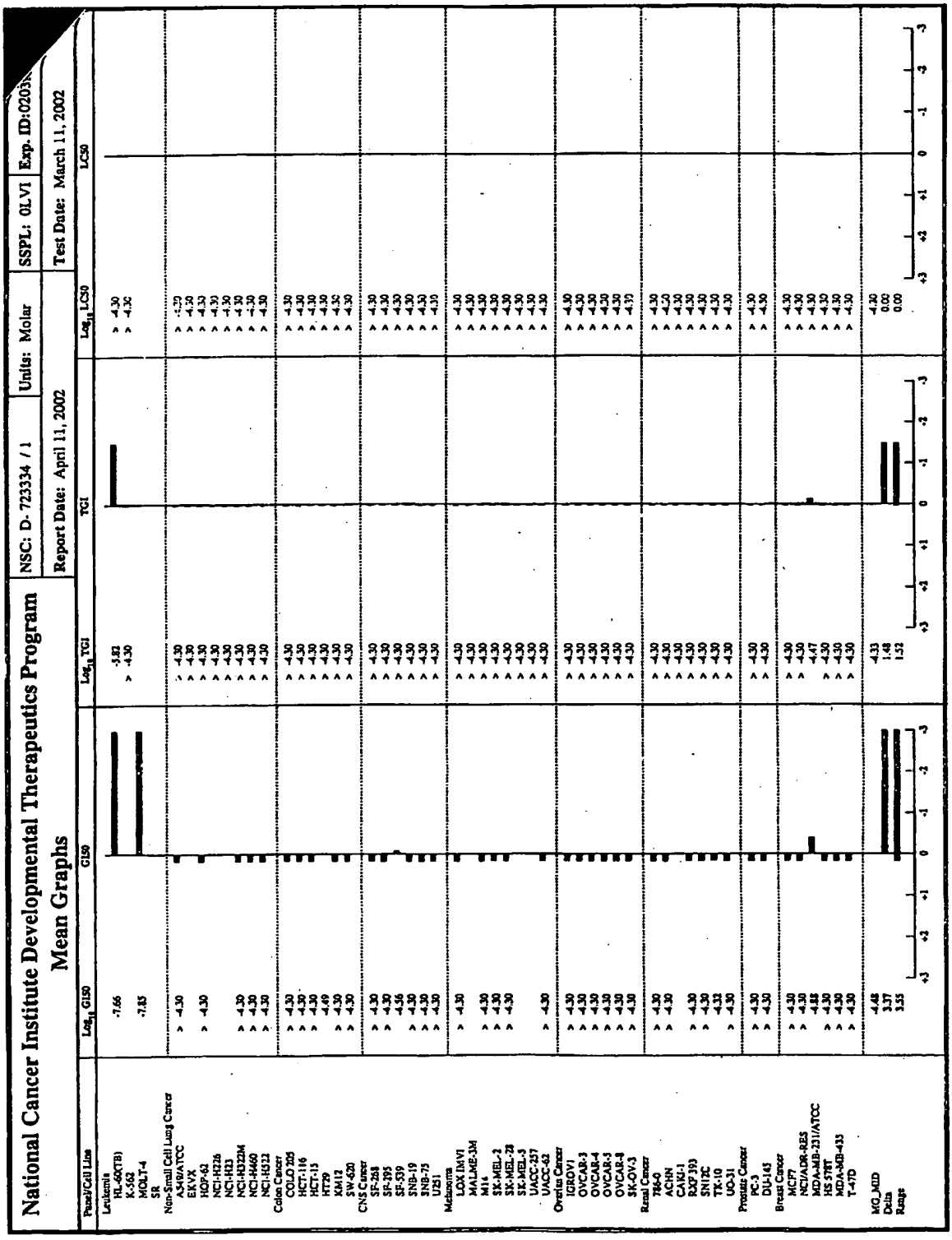
National Cancer Institute Developmental Therapeutics Program
Mean Graphs
NSC: D- 723334 / 1
Units: Molar
SSPL: 0LV1
Exp. ID:0203
Report Date: April 11, 2002
Test Date: March 11, 2002
Panel/Cell Line
Log10 GI50
Log10 TGI
Log10 LC50
GI50
TGI
LC50
Leukemia
HL-60(TB) -7.66 > -4.30 > -4.30
K-562 -5.83 > -4.30 > -4.30
MOLT-4 -7.85
SR
Non-Small Cell Lung Cancer
A549/ATCC > -4.30 > -4.30 > -4.30
EKVX > -4.30 > -4.30 > -4.30
HOP-62 > -4.30 > -4.30 > -4.30
NCI-H226 > -4.30 > -4.30 > -4.30
NCI-H23 > -4.30 > -4.30 > -4.30
NCI-H322M > -4.30 > -4.30 > -4.30
NCI-H460 > -4.30 > -4.30 > -4.30
NCI-H522 > -4.30 > -4.30 > -4.30
Colon Cancer
COLO 205 > -4.30 > -4.30 > -4.30
HCT-116 > -4.30 > -4.30 > -4.30
HCT-15 > -4.30 > -4.30 > -4.30
HT29 > -4.30 > -4.30 > -4.30
KM12 > -4.30 > -4.30 > -4.30
SW-620 > -4.30 > -4.30 > -4.30
CNS Cancer
SF-268 > -4.30 > -4.30 > -4.30
SF-295 > -4.30 > -4.30 > -4.30
SF-539 > -4.30 > -4.30 > -4.30
SNB-19 > -4.30 > -4.30 > -4.30
SNB-75 > -4.30 > -4.30 > -4.30
U251 > -4.30 > -4.30 > -4.30
Melanoma
LOX IMVI > -4.30 > -4.30 > -4.30
MALME-3M > -4.30 > -4.30 > -4.30
M14 > -4.30 > -4.30 > -4.30
SK-MEL-2 > -4.30 > -4.30 > -4.30
SK-MEL-28 > -4.30 > -4.30 > -4.30
SK-MEL-5 > -4.30 > -4.30 > -4.30
UACC-257 > -4.30 > -4.30 > -4.30
UACC-62 > -4.30 > -4.30 > -4.30
Ovarian Cancer
IGROV1 > -4.30 > -4.30 > -4.30
OVCAR-3 > -4.30 > -4.30 > -4.30
OVCAR-4 > -4.30 > -4.30 > -4.30
OVCAR-5 > -4.30 > -4.30 > -4.30
OVCAR-8 > -4.30 > -4.30 > -4.30
SK-OV-3 > -4.30 > -4.30 > -4.30
Renal Cancer
786-0 > -4.30 > -4.30 > -4.30
ACHN > -4.30 > -4.30 > -4.30
CAKI-1 > -4.30 > -4.30 > -4.30
RXF 393 > -4.30 > -4.30 > -4.30
SN12C > -4.30 > -4.30 > -4.30
TK-10 > -4.30 > -4.30 > -4.30
UO-31 > -4.30 > -4.30 > -4.30
Prostate Cancer
PC-3 > -4.30 > -4.30 > -4.30
DU-145 > -4.30 > -4.30 > -4.30
Breast Cancer
MCF7 > -4.30 > -4.30 > -4.30
NCI/ADR-RES > -4.30 > -4.47 > -4.30
MDA-MB-231/ATCC > -4.88 > -4.30 > -4.30
HS 578T > -4.30 > -4.30 > -4.30
MDA-MB-435 > -4.30 > -4.30 > -4.30
T-47D > -4.30 > -4.30 > -4.30
MG_MID -4.46 -4.33 -4.30
Delta 3.37 1.48 0.00
Range 3.55 1.52 0.00
+3 +2 +1 0 -1 -2 -3

Fig. 14

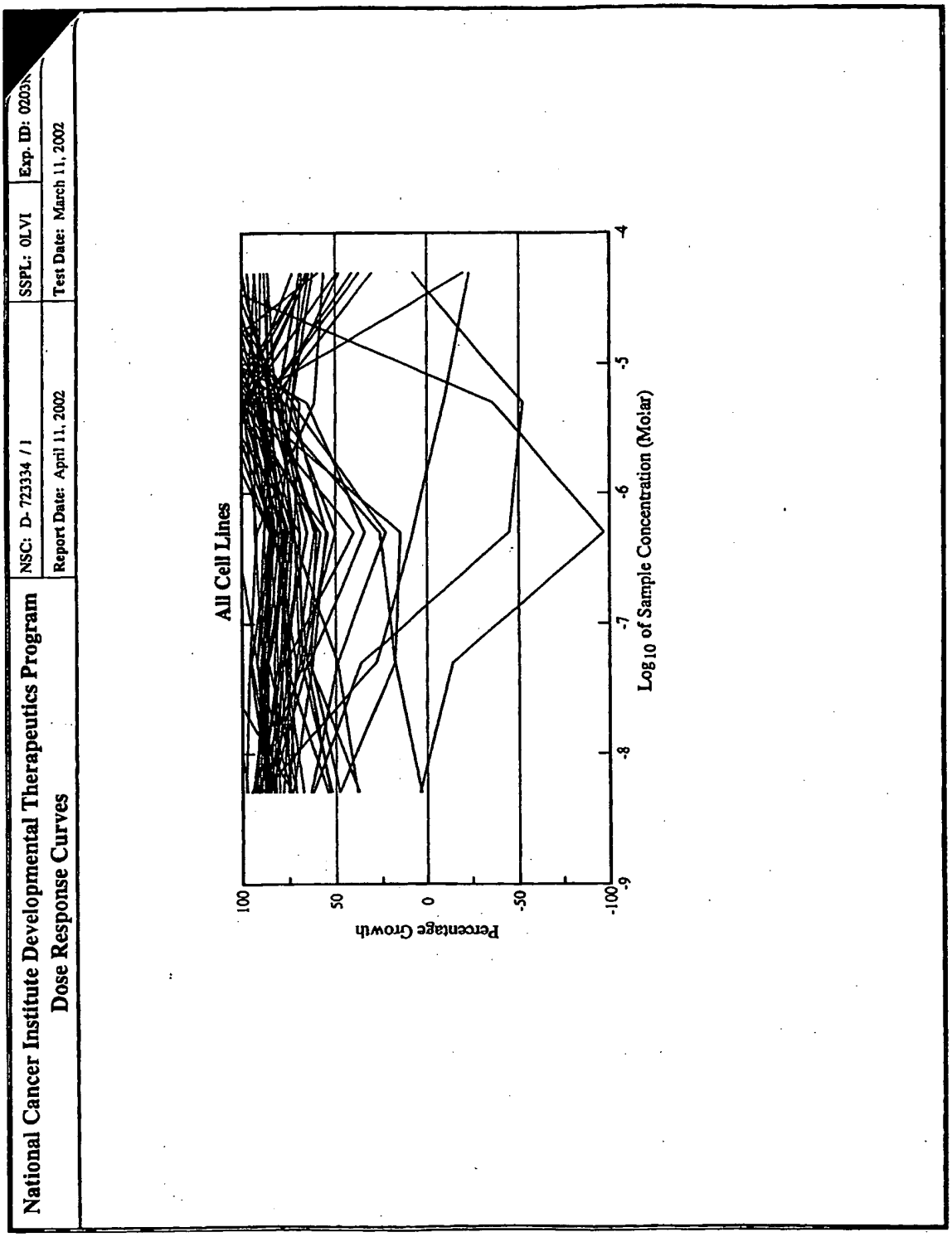
National Cancer Institute Developmental Therapeutics Program
Dose Response Curves
NSC: D-723334 / 1
Report Date: April 11, 2002
SSPL: 0LVI
Test Date: March 11, 2002
Exp. ID: 0203
All Cell Lines
Percentage Growth
100
50
0
-50
-100
-4
-5
-6
-7
-8
-9
Log10 of Sample Concentration (Molar)

Fig. 15

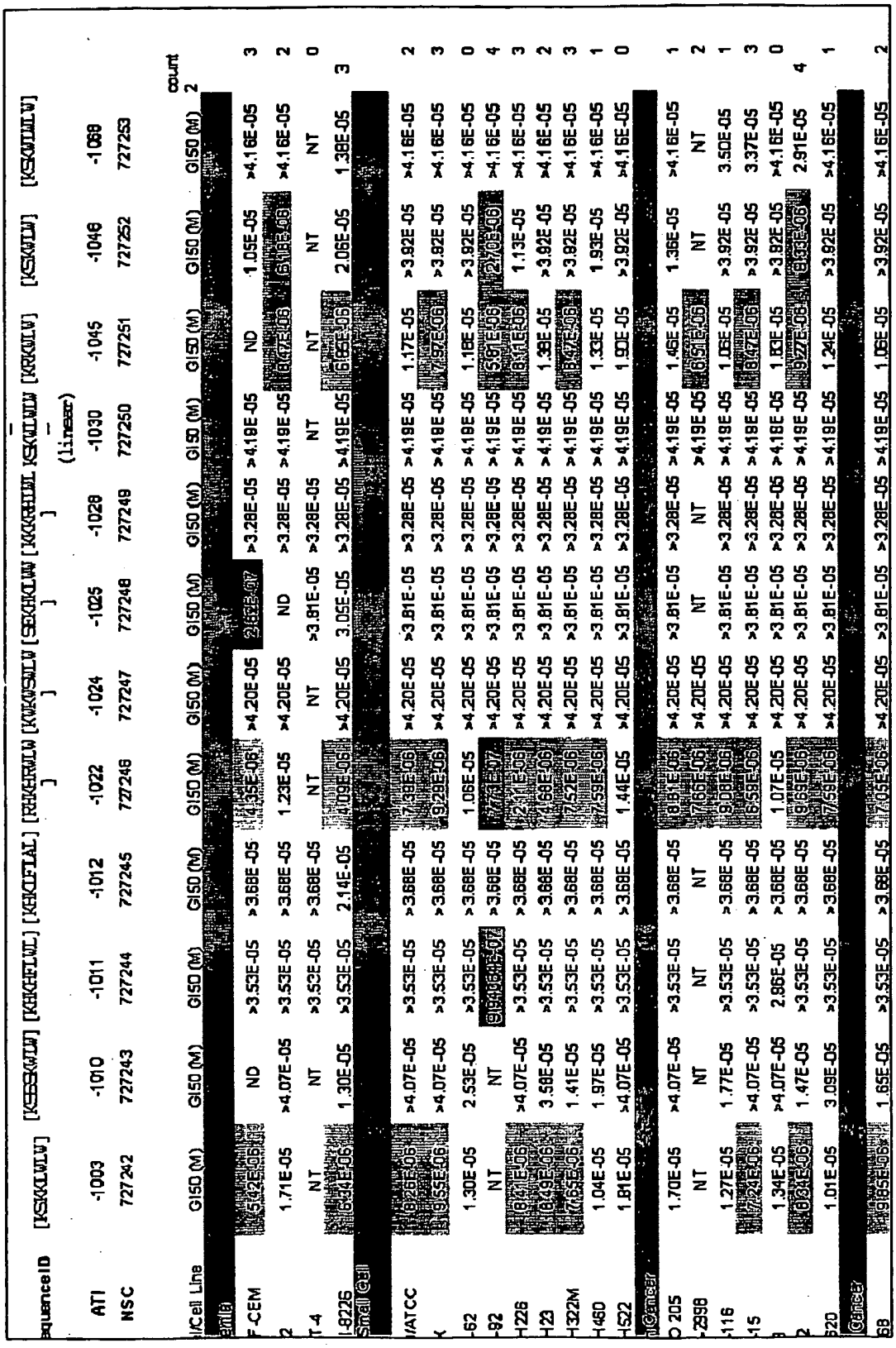

Fig. 16A

SF-539	1.01E-05	1.25E-05	1.71E-05	>3.68E-05	7.66E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.74E-06	1.45E-05	>4.16E-05	2
SNB-19	1.65E-05	>4.07E-05	>3.53E-05	>3.68E-05	7.05E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.10E-05	>3.92E-05	>4.16E-05	1
SNB-75	2.77E-05	>4.07E-05	>3.53E-05	>3.68E-05	1.63E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	2.38E-05	>3.92E-05	>4.16E-05	0
U251	1.14E-05	1.13E-05	>3.53E-05	>3.68E-05	7.12E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	9.03E-06	2.14E-05	2.77E-05	2
Melanoma													
LOX IMVI	1.20E-05	2.98E-05	>3.53E-05	>3.68E-05	8.70E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.27E-05	>3.92E-05	>4.16E-05	1
MALME-3M	9.92E-06	2.05E-05	>3.53E-05	>3.68E-05	1.61E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.15E-05	1.00E-05	>4.16E-05	1
M14	1.19E-05	>4.07E-05	>3.53E-05	>3.68E-05	1.19E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.04E-05	2.91E-05	>4.16E-05	0
SK-MEL-2	3.50E-05	>4.07E-05	>3.53E-05	>3.68E-05	3.27E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	>3.62E-05	>3.92E-05	>4.16E-05	0
SK-MEL-28	1.42E-05	2.88E-05	>3.53E-05	>3.68E-05	1.41E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.58E-05	>3.92E-05	>4.16E-05	0
SK-MEL-5	1.37E-05	2.37E-05	>3.53E-05	>3.68E-05	1.08E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.63E-06	1.46E-05	>4.16E-05	1
UACC-257	1.22E-05	>4.07E-05	>3.53E-05	>3.68E-05	8.54E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	9.92E-06	3.48E-05	>4.16E-05	2
UACC-62	8.79E-06	1.09E-05	>3.53E-05	>3.68E-05	7.93E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.55E-06	1.38E-05	>4.16E-05	3
Ovarian Cancer													
IGROV1	1.38E-05	>4.07E-05	>3.53E-05	>3.68E-05	9.76E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.48E-05	1.87E-05	>4.16E-05	1
OVCAR-3	9.07E-06	>4.07E-05	>3.53E-05	>3.68E-05	8.07E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.18E-05	>3.92E-05	>4.16E-05	2
OVCAR-4	1.38E-05	>4.07E-05	>3.53E-05	>3.68E-05	1.11E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.54E-05	>3.92E-05	>4.16E-05	0
OVCAR-5	1.81E-05	>4.07E-05	>3.53E-05	>3.68E-05	3.23E-05	>4.20E-05	>3.81E-05	>3.28E-05	1.80E-05	1.51E-05	>3.92E-05	>4.16E-05	0
OVCAR-8	1.94E-05	>4.07E-05	>3.53E-05	>3.68E-05	7.19E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.12E-06	>3.92E-05	>4.16E-05	3
SK-OV-3	1.05E-05	>4.07E-05	>3.53E-05	>3.68E-05	6.47E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.18E-05	>3.92E-05	>4.16E-05	1
Renal Cancer													
786-0	1.26E-05	2.23E-05	>3.53E-05	>3.68E-05	1.04E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	9.41E-06	1.83E-05	2.47E-05	1
A498	1.57E-05	3.16E-05	>3.53E-05	>3.68E-05	1.36E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.21E-05	>3.92E-05	>4.16E-05	0
ACHN	8.47E-06	>4.07E-05	>3.53E-05	>3.68E-05	8.07E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.91E-06	>3.92E-05	>4.16E-05	3
CAKI-1	9.70E-06	>4.07E-05	>3.53E-05	>3.68E-05	8.54E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.20E-05	>3.92E-05	>4.16E-05	2
RXF 393	9.17E-06	3.36E-05	>3.53E-05	>3.68E-05	9.22E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.22E-05	2.53E-05	>4.16E-05	2
SN12C	7.52E-06	>4.07E-05	>3.53E-05	>3.68E-05	8.41E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	8.03E-06	>3.92E-05	>4.16E-05	3
TK-10	8.64E-06	>4.07E-05	>3.53E-05	>3.68E-05	1.28E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	9.41E-06	>3.92E-05	>4.16E-05	2
UO-31	1.58E-05	>4.07E-05	>3.53E-05	>3.68E-05	1.38E-05	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	1.56E-05	>3.92E-05	>4.16E-05	0
Prostate Cancer													
PC-3	1.36E-07	>4.07E-05	1.54E-05	>3.68E-05	2.75E-05	ND	>3.81E-05	>3.28E-05	>4.19E-05	5.96E-06	>3.92E-05	>4.16E-05	3
DU-145	8.64E-06	>4.07E-05	>3.53E-05	>3.68E-05	6.71E-06	>4.20E-05	>3.81E-05	>3.28E-05	>4.19E-05	7.75E-06	>3.92E-05	>4.16E-05	3

Fig. 16B

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<151> 2002-05-06

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<221> SITE
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<223> Xaa = D-Leu.

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Xaa Lys Xaa Trp Xaa Trp Xaa Trp
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Xaa Gly Xaa Trp Xaa Trp Xaa Trp
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<221> SITE
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<221> SITE
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Gln Xaa Xaa Xaa Xaa Xaa Trp Xaa
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<223> Xaa = L-hydrophobic.

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<221> SITE
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<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-Phe.

<400> 42
Lys Xaa Trp Xaa Trp Xaa Trp Xaa
1 5

<210> 43
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Trp.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-His.

<400> 43
Lys Xaa Trp Xaa Trp Xaa Trp Xaa
1 5

<210> 44
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Trp.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<400> 44
Lys Xaa Trp Xaa Trp Xaa Lys Xaa
1 5

<210> 45
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 45
Lys Xaa Lys Xaa Ala Xaa Ala Xaa
1 5

<210> 46
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 46
Lys Xaa Lys Xaa Ala Xaa Trp Xaa
1 5

<210> 47
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 47
Lys Xaa Lys Xaa Phe Xaa Ala Xaa
1 5

<210> 48
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 48
Lys Xaa Lys Xaa Phe Xaa Phe Xaa
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2

<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 49
Lys Xaa Lys Xaa Phe Xaa Trp Xaa
1 5

<210> 50
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 50
Lys Xaa Lys Xaa Trp Xaa Trp Xaa
1 5

<210> 51
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Lys.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Phe.

<400> 51
Lys Xaa Lys Xaa Leu Xaa Leu Xaa
1 5

<210> 52
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 52
Lys Xaa Lys Xaa Phe Xaa Phe Xaa
1 5

<210> 53
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8

<223> Xaa = D-Leu.

<400> 53
Lys Xaa Lys Xaa Ala Xaa Ala Xaa
1 5

<210> 54
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 54
Lys Xaa Lys Xaa Phe Xaa Phe Xaa
1 5

<210> 55
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 55
Lys Xaa Lys Xaa Phe Xaa Phe Xaa
1 5

<210> 56
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 56
Lys Xaa Lys Xaa Gly Xaa Gly Xaa
1 5

<210> 57
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 57
Lys Xaa Lys Xaa Ile Xaa Ile Xaa

1 5

<210> 58
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 58
Lys Xaa Lys Xaa Ile Xaa Ile Xaa
1 5

<210> 59
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<221> SITE
<222> 5, 7
<223> Xaa = D-Nle.

<400> 59
Lys Xaa Lys Xaa Xaa Xaa Xaa Xaa
1 5

<210> 60
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 60
Lys Xaa Lys Xaa Val Xaa Val Xaa
1 5

<210> 61
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 61
Lys Xaa Lys Xaa Trp Xaa Trp Xaa

1 5

<210> 62
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 62
Lys Xaa Lys Xaa Tyr Xaa Tyr Xaa
1 5

<210> 63
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<221> SITE
<222> 3
<223> Xaa = Ornithine.

<400> 63
Lys Xaa Xaa Xaa Trp Xaa Trp Xaa
1 5

<210> 64
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Thr.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 64
Lys Xaa Lys Xaa Ala Xaa Ala Xaa
1 5

<210> 65
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Thr.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 65
Lys Xaa Lys Xaa Phe Xaa Phe Xaa

1 5

<210> 66
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Thr.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 66
Lys Xaa Lys Xaa Trp Xaa Trp Xaa
1 5

<210> 67
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Trp.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-Ser.

<400> 67
Lys Xaa Trp Xaa Trp Xaa Lys Xaa
1 5

<210> 68
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 68
Gln Xaa Phe Xaa Leu Xaa Trp Xaa
1 5

<210> 69
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 69
Gln Xaa Leu Xaa Leu Xaa Trp Xaa

1 5

<210> 70
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 70
Gln Xaa Val Xaa Leu Xaa Trp Xaa
1 5

<210> 71
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 71
Gln Xaa Trp Xaa Leu Xaa Trp Xaa
1 5

<210> 72
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 72
Lys Xaa Lys Xaa Phe Xaa Trp Xaa
1 5

<210> 73
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 73
Lys Xaa Lys Xaa Trp Xaa Trp Xaa
1 5

<210> 74
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-His.

<400> 74
Lys Xaa Trp Xaa Trp Xaa Lys Xaa
1 5

<210> 75
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-Ser.

<400> 75
Lys Xaa Trp Xaa Trp Xaa Lys Xaa
1 5

<210> 76
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4
<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 76
Lys Xaa Lys Xaa Phe Xaa Phe Xaa
1 5

<210> 77
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4

<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 77
Lys Xaa Lys Xaa Trp Xaa Trp Xaa
1 5

<210> 78
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4
<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 78
Lys Xaa Ser Xaa Ala Xaa Ala Xaa
1 5

<210> 79
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6
<223> Xaa = D-Lys.

<221> SITE
<222> 8
<223> Xaa = D-Leu.

<400> 79
Lys Xaa Ser Xaa Phe Xaa Phe Xaa
1 5

<210> 80
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4
<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 80
Lys Xaa Ser Xaa Val Xaa Val Xaa
1 5

<210> 81

<400> 81
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<210> 82
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4
<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 82
Lys Xaa Ser Xaa Trp Xaa Trp Xaa
1 5

<210> 83
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4
<223> Xaa = D-Lys.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 83
Lys Xaa Ser Xaa Tyr Xaa Tyr Xaa
1 5

<210> 84
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<400> 84
Lys Xaa Trp Xaa Trp Xaa Arg Xaa
1 5

<210> 85
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8

<223> Xaa = D-Trp.

<400> 85
Lys Xaa Lys Xaa Lys Xaa Leu Xaa
1 5

<210> 86
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 86
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 87
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp.

<400> 87
Lys Xaa Gln Xaa Lys Xaa Leu Xaa
1 5

<210> 88
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His.

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp.

<400> 88
Lys Xaa Ser Xaa Lys Xaa Leu Xaa
1 5

<210> 89
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Asn.

<221> SITE
<222> 6, 8
<223> Xaa = D-Phe.

<400> 89
Lys Xaa Gln Xaa Lys Xaa Trp Xaa

1 5

<210> 90
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Asn.

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 90
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 91
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Ser.

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp.

<400> 91
Lys Xaa Lys Xaa Lys Xaa Leu Xaa
1 5

<210> 92
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Ser.

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp.

<400> 92
Lys Xaa Gln Xaa Lys Xaa Leu Xaa
1 5

<210> 93
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Ser.

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp.

<400> 93
Lys Xaa Ser Xaa Lys Xaa Leu Xaa
1 5

<210> 94
<211> 8

<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-Tyr.

<221> SITE
<222> 6, 8
<223> Xaa = D-Asn.

<400> 94
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 95
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4, 6, 8
<223> Xaa = D-Ala.

<400> 95
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 96
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 6
<223> Xaa = D-Ala.

<221> SITE
<222> 4, 8
<223> Xaa = D-Asn.

<400> 96
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 97
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 6
<223> Xaa = D-Ala.

<221> SITE
<222> 4, 8
<223> Xaa = D-Phe.

<400> 97
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 98
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4

<223> Xaa = D-Phe.

<221> SITE
<222> 6, 8
<223> Xaa = D-Asn.

<400> 98
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 99
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Phe.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<221> SITE
<222> 8
<223> Xaa = D-Trp.

<400> 99
Lys Xaa Trp Xaa Trp Xaa His Xaa
1 5

<210> 100
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 8
<223> Xaa = D-Phe.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<400> 100
Lys Xaa Trp Xaa Trp Xaa Lys Xaa
1 5

<210> 101
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 8
<223> Xaa = D-Trp.

<221> SITE
<222> 6
<223> Xaa = D-His.

<400> 101
Lys Xaa Lys Xaa Leu Xaa Leu Xaa
1 5

<210> 102
<211> 8
<212> PRT
<213> Artificial Sequence

<220>

<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Phe.

<400> 102
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
1 5

<210> 103
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 103
Lys Xaa Lys Xaa His Xaa His Xaa
1 5

<210> 104
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 104
Lys Xaa Lys Xaa Gln Xaa Gln Xaa
1 5

<210> 105
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 105
Lys Xaa Lys Xaa Arg Xaa Arg Xaa
1 5

<210> 106
<211> 8
<212> PRT
<213> Artificial Sequence

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<221> SITE
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<223> Xaa = D-Ser.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Leu.

<400> 106
Lys Xaa Lys Xaa Arg Xaa Arg Xaa
1 5

<210> 107
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4, 6, 8
<223> Xaa = D-Trp.

<400> 107
Lys Xaa Lys Xaa Ser Xaa Leu Xaa
1 5

<210> 108
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<221> SITE
<222> 2, 4
<223> Xaa = D-Tyr.

<221> SITE
<222> 6, 8
<223> Xaa = D-Asn.

<400> 108
Lys Xaa Gln Xaa Lys Xaa Trp Xaa
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<210> 109
<211> 8
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<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
<222> 3
<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 109
Xaa Arg Xaa Trp Xaa Glu Xaa Trp
1 5

<210> 110
<211> 8
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<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
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<221> SITE
<222> 3

<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 110
Xaa Arg Xaa Trp Xaa His Xaa Trp
1 5

<210> 111
<211> 8
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<220>
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<221> SITE
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<221> SITE
<222> 3
<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 111
Xaa Arg Xaa Trp Xaa Lys Xaa Trp
1 5

<210> 112
<211> 8
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<220>
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<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
<222> 3
<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 112
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<210> 113
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<220>
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<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
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<223> Xaa = D-Trp.

<221> SITE
<222> 6
<223> Xaa = D-Tyr.

<400> 113
Lys Xaa Val Xaa Ala Xaa
1 5

<210> 114
<211> 6

<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Trp.

<221> SITE
<222> 4
<223> Xaa = D-Leu.

<221> SITE
<222> 6
<223> Xaa = D-Arg.

<400> 114
Lys Xaa Ile Xaa Ala Xaa
1 5

<210> 115
<211> 6
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<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Leu.

<400> 115
Arg Xaa Trp Xaa Trp Xaa
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<210> 116
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4, 6
<223> Xaa = D-Trp.

<400> 116
Lys Xaa Gln Xaa Leu Xaa
1 5

<210> 117
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
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<221> SITE
<222> 2
<223> Xaa = D-Glu.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 117
Lys Xaa Lys Xaa Leu Xaa
1 5

<210> 118
<211> 6
<212> PRT
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<220>

<223> A peptide containing D amino acids.

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<223> Xaa = D-Glu.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 118
Lys Xaa Gln Xaa Leu Xaa
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<210> 119
<211> 6
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<220>
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<221> SITE
<222> 2
<223> Xaa = D-His.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 119
Lys Xaa Lys Xaa Leu Xaa
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<210> 120
<211> 6
<212> PRT
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<220>
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<221> SITE
<222> 2
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<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 120
Lys Xaa Gln Xaa Leu Xaa
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<210> 121
<211> 6
<212> PRT
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<222> 2
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<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 121
Lys Xaa Lys Xaa Leu Xaa
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<210> 122
<211> 6
<212> PRT
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<223> Xaa = D-Lys.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 122
Lys Xaa Gln Xaa Leu Xaa
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<210> 123
<211> 6
<212> PRT
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<221> SITE
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<223> Xaa = D-Asn.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 123
Lys Xaa Gln Xaa Leu Xaa
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<210> 124
<211> 6
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<220>
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<221> SITE
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<223> Xaa = D-Gln.

<221> SITE
<222> 4, 6
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<400> 124
Lys Xaa Gln Xaa Leu Xaa
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<210> 125
<211> 6
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<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 125
Lys Xaa Lys Xaa Leu Xaa
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<210> 126
<211> 6
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<221> SITE
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<223> Xaa = D-Ser.

<221> SITE
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<400> 126
Lys Xaa Lys Xaa Leu Xaa
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<211> 6
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<220>
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<221> SITE
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<223> Xaa = D-Val.

<400> 127
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<210> 128
<211> 6
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<221> SITE
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<223> Xaa = D-Phe.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 128
Lys Xaa Lys Xaa Leu Xaa
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<210> 129
<211> 6
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<221> SITE
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<223> Xaa = D-Phe.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 129
Lys Xaa Gln Xaa Leu Xaa
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<221> SITE
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<223> Xaa = D-Ser.

<221> SITE
<222> 6

<223> Xaa = D-Thr.

<400> 130
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<221> SITE
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<223> Xaa = D-Ser.

<221> SITE
<222> 6
<223> Xaa = D-Thr.

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Lys Xaa Gln Xaa Arg Xaa
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<210> 132
<211> 6
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<221> SITE
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<221> SITE
<222> 4
<223> Xaa = D-Arg.

<221> SITE
<222> 6
<223> Xaa = D-Thr.

<400> 132
Lys Xaa Ile Xaa Ile Xaa
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<210> 133
<211> 8
<212> PRT
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<220>
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<221> SITE
<222> 2
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6, 8
<223> Xaa = D-Trp.

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Arg Xaa Arg Xaa Leu Xaa Leu Xaa
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<211> 8
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<220>
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<221> SITE
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<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 134
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<210> 135
<211> 8
<212> PRT
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<220>
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<221> SITE
<222> 3
<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 135
Xaa Arg Xaa Trp Xaa Trp Xaa Trp
1 5

<210> 136
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<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
<222> 3
<223> Xaa = D-Lys.

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu.

<400> 136
Xaa Arg Xaa Trp Xaa Trp Xaa Trp
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<210> 137
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<221> SITE
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<221> SITE
<222> 3
<223> Xaa = D-Arg.

<221> SITE
<222> 5
<223> Xaa = D-Lys.

<400> 137
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<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
<222> 5
<223> Xaa = D-Lys.

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1 5

<210> 139
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<212> PRT
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<220>
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<221> SITE
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<221> SITE
<222> 4, 6
<223> Xaa = D-Trp(Me)

<221> SITE
<222> 8
<223> Xaa = D-Trp.

<400> 139
Arg Xaa Lys Xaa Leu Xaa Leu Xaa
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<210> 140
<211> 8
<212> PRT
<213> Artificial Sequence

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<221> SITE
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<223> Xaa = D-Gln.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp(Me)

<221> SITE
<222> 8
<223> Xaa = D-Trp.

<400> 140
Lys Xaa Arg Xaa Leu Xaa Leu Xaa
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<210> 141
<211> 6
<212> PRT
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<221> SITE
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<223> Xaa = D-Arg.

<221> SITE
<222> 3, 5

<223> Xaa = D-Leu.

<400> 141
Xaa Lys Xaa Trp Xaa Trp
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<210> 142
<211> 8
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<213> Artificial Sequence

<220>
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<221> SITE
<222> 2, 8
<223> Xaa = D-Arg.

<221> SITE
<222> 4, 6
<223> Xaa = D-Trp.

<400> 142
Gln Xaa Trp Xaa Leu Xaa Trp Xaa
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<210> 143
<211> 8
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<220>
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<221> SITE
<222> 1
<223> Xaa = D-Lys.

<221> SITE
<222> 3, 5, 7
<223> Xaa = D-Leu.

<400> 143
Xaa Glu Xaa Trp Xaa Trp Xaa Trp
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<210> 144
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<220>
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<221> SITE
<222> 2, 4
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<221> SITE
<222> 6, 8
<223> Xaa = D-Leu.

<400> 144
Trp Xaa Lys Xaa Trp Xaa Trp Xaa
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<210> 145
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<220>
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<221> SITE
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<223> Xaa = D-Ser

<221> SITE
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<223> Xaa = D-Lys

<221> SITE
<222> 5, 7

<223> Xaa = D-Trp

<400> 145
Xaa Lys Xaa Leu Xaa Leu Xaa Lys
1 5

<210> 146
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<213> Artificial Sequence

<220>
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<221> SITE
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<223> Xaa = D-Gln

<221> SITE
<222> 3
<223> Xaa = D-Lys

<221> SITE
<222> 5, 7
<223> Xaa = D-Trp

<400> 146
Xaa Lys Xaa Leu Xaa Leu Xaa Lys
1 5

<210> 147
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<221> SITE
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<221> SITE
<222> 4, 6
<223> Xaa = D-Leu

<400> 147
Gln Xaa Arg Xaa Trp Xaa Trp Xaa
1 5

<210> 148
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<221> SITE
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<223> Xaa = D-Arg

<221> SITE
<222> 4
<223> Xaa = D-Leu

<221> SITE
<222> 8
<223> Xaa = D-Lys

<400> 148
His Xaa Trp Xaa Trp Xaa His Xaa
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<210> 149
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<221> SITE
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<223> Xaa = D-Ser

<221> SITE
<222> 4
<223> Xaa = D-Lys

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp

<400> 149
Lys Xaa Lys Xaa Leu Xaa Leu Xaa
1 5

<210> 150
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<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-His

<221> SITE
<222> 4
<223> Xaa = D-Arg

<221> SITE
<222> 6, 8
<223> Xaa = D-Leu

<400> 150
Lys Xaa Lys Xaa Trp Xaa Trp Xaa
1 5

<210> 151
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<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2, 4
<223> Xaa = D-His

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp

<400> 151
Arg Xaa Lys Xaa Arg Xaa Leu Xaa
1 5

<210> 152
<211> 8
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<220>
<223> A peptide containing D amino acids.

<221> SITE
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<223> Xaa = D-Gln

<221> SITE
<222> 4
<223> Xaa = D-Lys

<221> SITE
<222> 6, 8
<223> Xaa = D-Trp

<400> 152
Lys Xaa Lys Xaa Leu Xaa Leu Xaa
1 5

<210> 153
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<213> Artificial Sequence

<220>
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<221> SITE
<222> 1
<223> Xaa = D-Arg

<221> SITE
<222> 3
<223> Xaa = D-Lys

<221> SITE
<222> 5, 7
<223> Xaa = D-Trp

<400> 153
Xaa Ser Xaa Leu Xaa Leu Xaa Leu
1 5

<210> 154
<211> 8
<212> PRT
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<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 1, 3
<223> Xaa = D-Lys

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu

<400> 154
Xaa His Xaa His Xaa Trp Xaa Trp
1 5

<210> 155

<211> 8
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<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 1, 3
<223> Xaa = D-Lys

<221> SITE
<222> 5, 7
<223> Xaa = D-Leu

<400> 155
Xaa Gln Xaa Arg Xaa Trp Xaa Trp
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<210> 156
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
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<221> SITE
<222> 1, 5
<223> Xaa = D-Arg

<221> SITE
<222> 3
<223> Xaa = D-Lys

<221> SITE
<222> 7
<223> Xaa = D-Leu

<400> 156
Xaa His Xaa His Xaa Trp Xaa Trp
1 5

<210> 157
<211> 8
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<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 2
<223> Xaa = D-Glu

<221> SITE
<222> 4
<223> Xaa = D-His

<221> SITE
<222> 6
<223> Xaa = D-Leu

<221> SITE
<222> 8
<223> Xaa = D-Trp

<400> 157
Ser Xaa Lys Xaa Lys Xaa Trp Xaa
1 5

<210> 158
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A peptide containing D amino acids.

<221> SITE
<222> 1, 3
<223> Xaa = D-Lys

<221> SITE
<222> 5

<223> Xaa = D-His

<221> SITE
<222> 7
<223> Xaa = D-Trp

<400> 158
Xaa Lys Xaa Arg Xaa Leu Xaa Leu
1 5

<210> 159
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> A linear peptide.

<400> 159
Lys Ser Lys Trp Leu Trp Leu Trp
1 5

ARTIFACT SHEET

Enter artifact number below. Artifact number is application number + artifact type code (see list below) + sequential letter (A, B, C .). The first artifact folder for an artifact type receives the letter A, the second B, etc..
Examples: 59123456PA, 59123456PB, 59123456ZA, 59123456ZB

Indicate quantity of a single type of artifact received but not scanned. Create individual artifact folder/box and artifact number for each Artifact Type.

☐ CD(s) containing:
computer program listing ☐ Artifact Type Code: P
Doc Code: Computer
pages of specification
and/or sequence listing ☐
and/or table
Doc Code: Artifact Artifact Type Code: S
content unspecified or combined ☐
Doc Code: Artifact Artifact Type Code: U

☐ Stapled Set(s) Color Documents or B/W Photographs
Doc Code: Artifact Artifact Type Code: C

☐ Microfilm(s)
Doc Code: Artifact Artifact Type Code: F

☐ Video tape(s)
Doc Code: Artifact Artifact Type Code: V

☐ Model(s)
Doc Code: Artifact Artifact Type Code: M

☐ Bound Document(s)
Doc Code: Artifact Artifact Type Code: B

☐ Confidential Information Disclosure Statement or Other Documents marked Proprietary, Trade Secrets, Subject to Protective Order, Material Submitted under MPEP 724.02, etc.
Doc Code: Artifact Artifact Type Code X

☐ Other, description: _____
Doc Code: Artifact Artifact Type Code: Z